The Biology and Impact of *Neochetina* Weevils on Water Hyacinth, *Eichhornia crassipes* in Lake Victoria Basin, Kenya

By

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January 2004

DECLARATION

CANDIDATE

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ABSTRACT

Lake Victoria, like other large lakes in the world, is a shared resource and forms an international boundary between Kenya, Uganda and Tanzania. It is the second largest fresh water lake in the world with an area of 69,0000 Km², a convoluted shoreline of 4800Km and a volume of 2,700km³. The lake lies in one of the most populous regions in the world, serving as a source of livelihood for some 30 million people in Kenya, Uganda and Tanzania who depend on it for fisheries, transport, power generation, biodiversity conservation, tourism, recreation, agricultural, industrial and domestic needs. Current estimates put the annual fish catch between 400,000 and 500,000 metric tonnes generating some US\$300-400 million. In Kenya about 90% of the total fish landed is from this lake. Its fishery directly employs about 100,000 people but more than 2 million people are involved in other indirect activities.

The invasion of this strategic water body by the worlds worst aquatic weed, water hyacinth, *Eichhornia crassipes* in the early 1990s posed a serious challenge to the widely acknowledged biological control strategy. In its native range in the Amazonian Brazil, its natural enemies keep the weed in check. The most important of these are two Curculionidae weevils, *Neochetina eichhorniae* and *N. bruchi*. While control has been attained with remarkable success elsewhere in the world using these agents, no information is available on the biology, population dynamics and their impact on water hyacinth in the Lake Victoria Basin. These studies were therefore aimed at quantifying and filling these information gaps.

The mean fecundity of the two weevils was recorded as 290 and 237 eggs per female laid over a period of 16 weeks, with an adult longevity of 98 and 112 days for *Neochetina bruchi* and *N.eichhorniae* respectively. A two-way analysis of variance showed there was significant difference between the egg laying capacities of the two weevil species (p=0.002). The survival rate of the two species was significantly different (p<0.05) for all life stages except for larvae to pupa. There was no significant interaction between the species and the method of egg setting (p<0.05). These studies were conducted in an open laboratory at KARI, Kibos.

The impact of these weevils on water hyacinth was evaluated at 3 satellite ponds each in the riparian districts of Busia, Kisumu and Nyando. The parameters evaluated were fresh weight, number of ramettes, petioles, feeding scars, percent damaged petioles, laminar area and petiole length. The comparative field survival of the weevils was evaluated by recording the number of adults, eggs, larvae and pupae recovered at every site. A sample size of 18 plants was used at every site, with sampling conducted once every month for 12 calendar months at Okana (Kisumu) and Otho (Nyando). Data for Budalangi (Busia) was taken for six months only. The percent damaged petioles were consistently higher with an increase in the number of larvae (the most destructive stage) at all the sites.

These weevils are effective biological control agents and for maximum results, they should be used synergistically for the control of water hyacinth in the Lake Victoria Basin.

Key Words: Biological control, water hyacinth, *Neochetina*, weevils, Lake Victoria.

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ABBREVIATIONS AND ACRONYMS

a.s.l. above sea level

ACK Anglican Church of Kenya

ACTS African Centre for Technology Studies

ANOVA Analysis of Variance

BOD Biological Oxygen Demand

CABI Centre for Agricultural and Bioscience International

CAM Congo Air Mass CI Confidence Interval

COD Chemical Oxygen Demand

CSIRO Commonwealth Scientific and Industrial Research Organization

CT Controlled Temperature
EAC East African Community
ELD Egg to Larva Duration
EPD Egg to Pupa Duration
FES Free Egg Setting

GCI Galvanised Corrugated Iron GEF Global Environment Facility

GLM General Linear Model

GPS Geographic Positioning System

IDA International Development Assistance

IES Incision Egg Setting

IIBC International Institute of Biological Control IIE International Institute of Entomology

IITA International Institute for Tropical Agriculture IOBC International Organisation for Biological Control

IPM Integrated Pest Management
 ITCZ Intertropical Convergence Zone
 KARI Kenya Agricultural Research Institute
 KEPHIS Kenya Plant Health Inspectorate Service

KICK Kisumu Innovation Centre

LBDA Lake Basin Development Authority
LM Lower Midland Agroecological Zone

LPD Larva to Pupa Duration

LVEMP Lake Victoria Environmental Management Project
MCBI Marine Conservation Biology Institute, Washington DC

NARC National Agricultural Research Centre
NARO National Agricultural Research Organization
NEMA National Environment Management Authority

NAS National Academy of Sciences NFRC National Fibre Research Centre

ppm Parts Per Million

PPRI Plant Protection Research Institute

SE Standard Error SWB Small Water Bodies

UM Upper Midland Agroecological Zone
UNEP United Nations Environmental Programme

USA United States of America

USDA United States Department of Agriculture WMO World Meteorological Organization

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Professing themselves to be wise, without God, they became utter fools (Romans 1:22)

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TO GOD BE THE GLORY AND HONOUR

DEDICATION

To my dear uncle, guardian and mentor

Evans Chege Njoka BSc, MA (Wisconsin)

for his love, great inspiration and taking me to school

"Where is the wise man?

Where is the scholar?

Where is the philosopher of this age?

Has not God made foolish the wisdom of the world?"

1 Corinthians 1:20

CHAPTER ONE

1.0 Introduction

1.1 Background

All over the world and from time immemorial, large lakes have continued to play an important role in the livelihood and prosperity of mankind. Their uses include domestic and industrial water supply, irrigation, transport, fishing, waste assimilation, mineral extraction and recreation. Besides their special aesthetic appeal and sheer scenic beauty, most of the world's large lakes form international boundaries (Herdendorf, 1990).

Lake Victoria, with an area of 69,482 km² and a volume of 2,700 km³ is the second largest fresh water lake in the world, after North America's Lake Superior. The lake is located between latitude 0°20'North to 3°South and longitude 31°East to 34°52'East. It lies at about 900 m.a.s.l and is surrounded by relatively low-lying land averaging 1134 m.a.s.l. (Ongweny, 1979; Lewis *et al.*, 1988). The lake has a catchment area of 266,000km² distributed as follows: 44% in Tanzania, 22% in Kenya, 16% in Uganda, 11% in Rwanda and 7% in Burundi (Okidi *et al.*, 1982). The lake, tectonic in origin, measures about 337km at its longest and 240km at its widest with a mean depth of 40m and a maximum of 92m (Herdendorf, 1990). It is significantly stirred down to the bottom by winds most of the year, thus making the bottom waters well oxygenated and the nutrients well distributed. The lake has a high photosynthetic production and provides rich food for the fish, as typical of all eutrophic lakes (Wetzel, 1983).

The Lake's origins are still the subject of scientific dispute, but it seems likely that it is much more recent than the other great lakes of Eastern Africa. Many of the rivers now flowing east into the Lake, including Kagera, once flowed west, at least in the Miocene, Pliocene, and part of the Pleistocene eras (within the past 2million years), possibly eventually into the Nile system, and a more recent upthrust of the western side of the basin is thought to have reversed these rivers, and caused Lake Victoria to form by flowing eastwards. It is possible that the Lake could have formed as recently as 25,000 to 35,000 years ago, and recent evidence suggests it may have dried up completely between 10,000 and 14,000 years ago (Beadle, 1981).

Lake Victoria is the largest tropical lake in terms of size, species diversity, biomass and ecological variability (Herdendorf, 1990; Barel *et al.*, 1991). It is shared between Kenya (6%), Uganda (43%) and Tanzania (51%), with a total convoluted shoreline of around 4,828 km that encloses innumerable small shallow bays and inlets, many of which include swamps and wetlands (Hickling, 1961).

The lake lies in one of the most populous areas in the world, serving as a source of livelihood for some 30 million people in Kenya, Uganda and Tanzania (Ochumba, 1994). It is important to the riparian states for food, agricultural, industrial and domestic water supply, marine transport, biodiversity conservation, tourism and recreation. Ayot (1977) describes the lake as having provided water for large herds of cattle belonging to the Luo community who were initially cattle herders. Current estimates show that annual fish catch from Lake Victoria is between 400,000 - 500,000 metric tonnes generating some US\$ 300-400 million (Hirji and Carey, 1998). Over 90% of the total fish landed in Kenya comes from Lake Victoria (Ikiara, 1999). In Uganda, the lake, considered as the source of the Nile, provides the waters to generate electricity at the Owen Falls part of which has continued to be exported to Kenya since the 1950s. The Nile then continues as a lifeline to the Sudan Gezira and on to Egypt where it is sacred before emptying into the Mediterranean Sea.

The aquatic weed water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach in the family Pontederiaceae is an erect free floating, stoloniferous, perennial herb (Plate 1.1). It grows to one metre tall with buoyant leaves, which vary in size according to growth conditions. The bisexual flowers are lilac to lavender in colour with a central yellow area, borne on a single spike. Their beauty and appeal has encouraged intentional spread of the weed by man. The seed capsules contain up to 300 small long-lived seeds that sink on release. The seeds can remain viable for up to 30 years thus increasing the weed's capacity for regrowth and persistence in the environment (Manson and Manson 1958; Harley, 1994).



Plate 1. 1: A Close up Photo of the Water Hyacinth (Eichhornia crassipes) plant

In the past 100 years, water hyacinth has spread to many tropical and sub-tropical regions of the world from what is thought to be its origin in the Amazonian Brazil (Barrett and Forno, 1982). Water hyacinth is the world's most noxious aquatic weed and has been reported in at least 59 countries (Harley *et al.*, 1996). The weed's seriousness in its introduced range is not only as a result of its rapid growth rate through both vegetative reproduction and ability to re-infest via the seed bank or flood-borne plants but also due to lack of natural enemies in its introduced habitats (Abdel-Rahim and Tawfiq, 1984; Charudattan, 1986).

The present infestation of water hyacinth in Lake Victoria is suspected to have originated from the Rwandan highlands through River Kagera. It was first reported on the Ugandan side of the lake in 1988 (Thompson, 1991). The weed then quickly spread throughout the Lake establishing "Hotspots" (Figure 1). Other reports indicate it has been grown as an ornamental in East Africa since 1957 (Gopal, 1987). Although present in ponds and dams for some time in East Africa, the first place where water hyacinth was observed to have gone out of hand was on River Sigi, near Tanga in Tanzania in 1955. It also appeared on the nearby River Pangani in the neighbourhood of Korogwe and spread rapidly downstream (Ivens, 1982).

Water hyacinth and other invasive aquatic plants have many negative impacts on water bodies (Harley,1990). The first visible effect of a water hyacinth infestation is the quantity of biomass produced and the turbidity of the water body. Other impacts include disruption of marine transport, blocking of water outlet points, increase in the occurrence of water related diseases and loss of biodiversity. Different levels of water hyacinth infestation are shown in Plate 1.2.

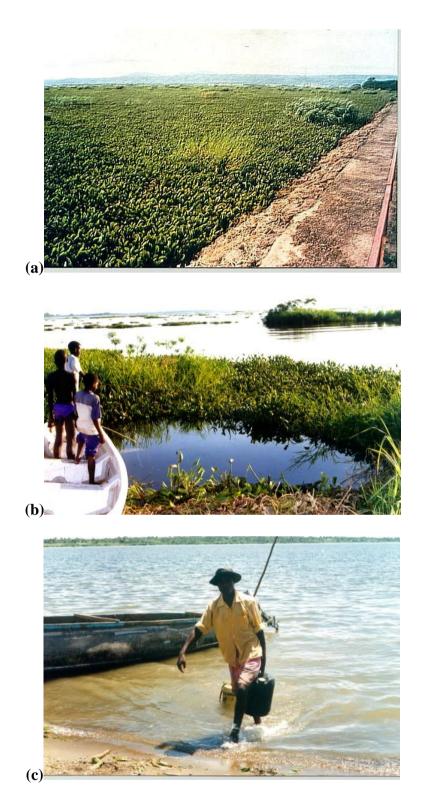


Plate 1. 2: Levels of Water Hyacinth infestation in Lake Victoria (a) Heavy, (b) Medium (c) No infestation

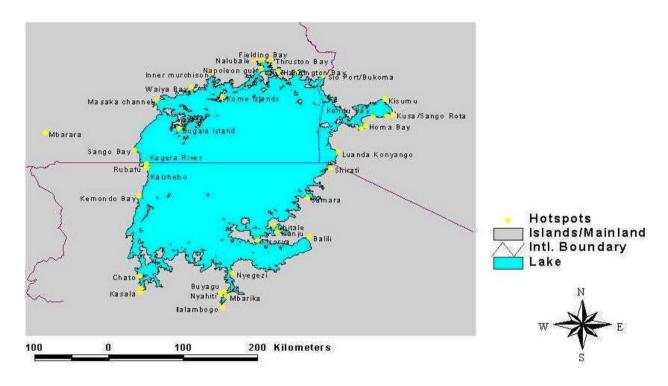


Figure 1. 1: Map of Lake Victoria indicating water hyacinth hotspots

Eco-climatic requirements for water hyacinth favour its current growth and proliferation in Lake Victoria, waterways, wetlands, dams, irrigation developments and hydroelectric schemes throughout the riparian states of Kenya, Uganda and Tanzania. The weed has had a profound socio-economic and environmental impact on the lake and the riparian communities. The socio-economic impacts include deleterious effects on lake transport, fishing activities, water supply and hydroelectric power generation and increase in the population of human and animal disease vectors. Environmental (ecological) impacts include the loss of biodiversity, eutrophication, evapotranspiration, deoxygenation and erosion of the lake's scenic beauty.

There are three main methods of control of this notorious weed namely physical, chemical and biological. Physical control involves both manual and mechanical removal, a tedious and time-consuming exercise, involving the use of huge and very expensive machinery and human labour. Chemical pesticides may have some perceived short-term success, especially in small-enclosed ponds, but their harmful effects on non-target organisms cannot be gainsaid. The riparian communities take the lake water in its raw form. The lake is also the source of the River Nile, a lifeline to the Sudanese and Egyptians who would suffer from chemicals used upstream.

There is limited understanding of the interactions between the different biological control agents. Heard and Winterton (2000) demonstrated the different requirements and hence potential for the damage caused by the two *Neochetina* weevils to be complimentary. However, relationships with the moths are less clear. The moth *Niphograpta albiguttalis* laid more eggs on water hyacinth plants where the leaf surface was damaged by *N. eichhorniae* (De Loach and Cordo, 1978) but according to Wright (1984) and Wright and Bourne (1986),

N. albiguttalis is often less abundant in areas where there are large populations of N. eichhorniae.

Good watershed management will help reduce the water hyacinth problem. High nutrient levels, brought about through such processes as deforestation, poor agricultural practices, urban runoff, and discharge of industrial and urban waste, promote fast growth of water hyacinth. Reducing nutrient inputs from these sources will slow the rate of growth and reduce spread of the weed. This will further improve the effectiveness of other control agents (Harley et al, 1996).

1.1 Statement of the Problem

Mason, (1983) remarks, "Lakes seem, on the scale of years or of human lifespan, permanent features of landscape, but they are geologically transitory, usually born of catastrophes, to mature and die quietly and imperceptibly". Since water hyacinth first appeared in Lake Victoria in 1988 on the Ugandan side (Njoka et al., 1988), it has continued to grow almost unchecked and was at its peak infestation estimated to occupy circa 6,000 ha on the Kenyan side (Ochiel and Njoka, 2001). The bulk of these mats are to be found clogging beaches, fish landing sites, water intake points, piers and the strategically important regional port of Kisumu. The lake is economically important not only to the riparian states but also to the Nile basin states of Sudan, Ethiopia and Egypt for agricultural and industrial development.

The invasion of Lake Victoria by water hyacinth has adversely affected the normal utilities of the lake thus deteriorating the communities' economic and health status, as well as causing adverse changes in the lake's water quality and biodiversity. The most adversely

affected part is the Winam Gulf of Lake Victoria and the numerous outlying ponds in the Lake Basin.

Control of water hyacinth is causing serious challenge to the riparian governments and communities around the Lake. Amongst the various control methods being considered, biological control stands out to be a major component of integrated pest management and a globally acceptable tool for sustainable management of this exotic and noxious weed. The use of both mechanical and chemical techniques are faced by numerous ecological and management problems making their application in Lake Victoria difficult or only applicable in selected areas.

One of the reasons for the rapid proliferation of water hyacinth is the lack of its natural enemies in Lake Victoria, which is not its centre of origin. This makes the weed more amenable to classical biological control methods. In its native home, the Amazonian Brazil, several natural enemies occur which have recently been imported into East Africa to be used against the weed. The problem is that limited research data is locally available for effective use of these agents.

Two weevils, *Neochetina bruchi* Hustache and *N. eichhorniae* Warner are presently being considered as candidates for biological control of water hyacinth control in Lake Victoria. Through the World Bank / Lake Victoria Environmental Management Project (LVEMP) support, the Kenya Agricultural Research Institute (KARI) is mandated to conduct research on water hyacinth in the Kenyan portion of the lake. However, the constraint facing successful implementation of this project is lack of data on the biology and life cycle of these insects to facilitate their mass rearing and timely release on water hyacinth.

Data is also lacking on their survival, establishment, population dynamics, and their impact on water hyacinth in the lake and in the outlying ponds, swamps and wetlands.

1.2 Justification of the Study

There exists a vast amount of research literature about Lake Victoria—fish species, plants and microorganisms, ecosystems diversity as well as fisheries related socio-economics (Okeyo-Owuor, 1999). However, no studies have been done on the current invasion of water hyacinth especially the potential, implications and impact of its exotic natural enemies, which were recently imported for its biological control. The Lake is faced with numerous ecological and socio-economic pressures of which the invasion by water hyacinth is a key player (Anon, 1996). The use of biological control to reduce the vast mats of water hyacinth now existing in the Winam Gulf and associated ponds in the Kenyan side of the lake will significantly ease this pressure. It will contribute to sustainable conservation of the lake's biodiversity and help improve livelihood in the region. The riparian countries through the World Bank funded LVEMP are currently implementing a sustainable management programme to alleviate the present environmental problems facing the lake. The research on control of water hyacinth is a major component coordinated by KARI, at its National Fibre Research Centre (NFRC), Kibos. Through this component, biological control deserves priority and therefore detailed research on the two imported phytophagous weevils, *Neochetina bruchi* and *N. eichhorniae* deserve attention.

Elsewhere biological control of water hyacinth using *Neochetina* weevils has been attempted with some success in the Sudan, Zimbabwe, Egypt and South Africa (Beshir and Bennett, 1985; Cilliers, 1991; Julien, 1992; Chikwenhere, 1994) and recently in Uganda's Lake

Kyoga (Ogwang' and Molo, 1997). Data still needs to be generated on their bionomics and performance in Lake Victoria and the surrounding water bodies. Sufficient data on the biology, mass rearing, life table, survival, establishment and performance on the weed under local conditions need to be collected. This is justifiable since such data will contribute significantly to the implementation of an integrated pest management strategy of the weed.

1.3 Overall Objective

To contribute to sustainable management of Lake Victoria and reduction of water hyacinth invasion menace by investigating the bionomics, survival and impact of the two exotic curculionidae weevils, *Neochetina bruchi* and *N. eichhorniae* on water hyacinth in the Lake Victoria basin, Kenya.

1.3.1 Specific Objectives

- i) To determine the biology and life cycle of *Neochetina* weevils and factors affecting their development under semi-controlled conditions.
- ii) To investigate the survival and development of *Neochetina* weevils under local field conditions.
- iii) To evaluate the impact of *Neochetina* weevils on the growth and proliferation of water hyacinth at different field sites.

1.3.2 Hypotheses

- i) The biology and life cycle of *Neochetina* weevils are not affected by local climatic factors particularly temperature.
- ii) The survival and development of *Neochetina* weevils is not affected by weather factors in the Winam Gulf.
- iii) Neochetina weevils are not effective biological control agents of water hyacinth in the Lake Victoria basin.

1.4 Scope of the study

The biology, survival and impact of *Neochetina* weevils on water hyacinth was studied in an uncontrolled laboratory and in the shores and small water bodies of the Winam Gulf, Kenya. Studies were not extended to the main lake due to the frequent movement of the weed in the open waters, which makes it difficult to evaluate plant and insect parameters. The studies under the uncontrolled laboratory conditions provided a near real field condition suitable to the practical application of these studies.

CHAPTER TWO

2.0 Literature Review

2.1 Overview

The aquatic weed water hyacinth is ranked among the top ten weeds worldwide and is one of the most successful colonisers in the plant kingdom (Holm et al, 1977). Its rapid growth, vegetative reproduction and ability to reinfest via the seed bank or flood borne plants have resulted in excessive infestations in Africa, southern Asia and the USA. The weed impairs water quality and quantity, transport, irrigation, hydroelectricity generation, water use and biodiversity (Gopal, 1987).

The weed's native range is in the Amazonian Brazil where natural enemies keep it in check. The invasion of Lake Victoria by this weed was first noticed on the Ugandan side in 1988 possibly through River Kagera (Njoka *et al.*, 1988; Thompson, 1991). The weed has continued to infest water bodies in the Lake Victoria Basin and beyond. It hampers water transport, blocks fish landing sites, impedes access by humans and livestock besides harbouring disease vectors. It is thus a threat to the environment and economy of the region.

Elsewhere in the world, the weed has been successfully managed using biological control agents mainly the two weevils of the genus *Neochetina*. Biological control is sustainable and environmental friendly while chemical and mechanical control measures are expensive and ineffective on all but small infestations (Julien *et al.*, 2001). Further, continuous use of chemicals has an adverse effect on non-targets and

is contrary to the Biodiversity Convention enshrined in Agenda 21 of the United Nations.

2.2 Water Hyacinth

2.2.1 Description and Taxonomy

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach is a perennial, herbaceous, free-floating freshwater plant originating from the Amazonian Brazil, South America. It belongs to the family Pontederiaceae, and was first described by C.F.P. Von Martius, an explorer of tropical South America in 1824 (Gopal, 1987). The family contains eight other genera and is widely distributed throughout the world being almost cosmopolitan today due to introduction by man. It reached botanical gardens in Europe even before Von Martius formally christened it, since von Humboldt, another famous explorer, had collected the water hyacinth from the banks of River Cuaca in present day Colombia in 1801 (Gopal, 1987). Seven other species of the genus *Eichhornia* are reported, six of which are native to South America and another one, *E. natans* is native to Africa. Only *E. crassipes* is regarded as a pantropical weed of fresh water lakes, rivers and canals. Natural enemies keep the African native species, *E. natans* under control just like the *E. crassipes* in South America (Gopal 1987; Julien *et al.*, 1999).

Water hyacinth shows considerable variation in both leaf and flower form. The petioles vary from long and relatively slender to swollen or bulbous. The shape of the petiole influences the amount of air contained and consequently the capacity to float. Slender petioles are typical of plants that occur within dense, crowded infestations, while

bulbous petioles characterise younger plants in open water or on the open-water margins of infestations (Julien *et al*, 2001).

Water hyacinth consists of a fibrous root system, a basal rhizome, elongated, buoyant petiole and a small simple leaf. The roots are adventitious, un-branched, darkly pigmented ending in a conspicuous root cap and may extend up to 3 metres. The leaves are comprised of a smooth, glossy, circular to kidney shaped lamina and a thick, spongy, parenchyma filled petiole. The plant is over 90% water with many air chambers within the petioles that enable it to float. Water hyacinth floats while all other members of the family Pontederiacea are rooted in the substrate (Manson and Manson, 1958; Gopal, 1987; Julien *et al.*, 2001).

Flowers are of three distinct types, differing in the relative length of styles within the single flowers. In the introduced range of the species, the form with styles of intermediate length predominates, the long-styled form occurs less frequently and the hypothesised short-styled form has not been recorded. Flowers are lavender in colour and borne on a terminal inflorescence bearing up to 60, but usually 8-15 flowers (Barrett, 1977; Barrett and Forno, 1982).

2.2.2 Reproduction and productivity

The plant reproduces sexually and by vegetative propagules. The flowers can self-fertilise. The fruit is a thin walled capsule containing up to 450 seeds which sink into the water upon release, posing serious re-infestation problems in otherwise cleared areas. The seeds can remain viable for up to 30 years. Vegetative reproduction is a common form of propagation and is largely responsible for the rapid increase and spread of water

hyacinth into new areas. The daughter plants produced from the horizontal stolons develop roots and eventually separate from the mother plant following decay or breakage of the connecting stolon. Currents, winds, fishing nets and watercraft readily distribute these plants (Sculthorpe, 1971). Under favourable conditions a single plant can develop into a substantial infestation in a very short time. In fact, the plants can double its progeny in 6-15 days (Bakar *et al.*, 1984; Kumar *et al.*, 1985). Three plants may produce 3,000 new plants in 50 days (Aston, 1973) or 140 million plants every year, enough to cover 140 hectares with a fresh weight of 28,000 tonnes (Otieno and Wangila, 1993). In the Congo, two plants were observed to produce 1,200 daughter plants in four months, and in a dense infestation, the mass of vegetation can be thick enough to support the weight of a man (Ivens, 1982).

Eichhornia crassipes is one of the most productive plants on earth. Reports of productivity by this aquatic plant include 173, 123 and 106 tons ha⁻¹ yr⁻¹ in Florida, Guyana and Indonesia respectively (Gopal, 1987). Wolverton and McDonald (1978) reported a growth rate of 800 kg ha⁻¹ day⁻¹. Productivity tends to be less with decreasing temperature and greater in nutrient rich waste waters (Haider, 1984; John, 1984). A stand of 19.7 tons ha⁻¹ (dry weight) containing 2415 kg N and 465 kg P ha⁻¹ was reported in India (Baruah, 1984). Batanouny and El-fiky (1984) reported a 30-fold increase in biomass, which produced 43 offsets (vegetative propagules) over 50 days. The coverage of water hyacinth in the Curug Reservoir in Java changed from 3 to 48 hectares in 50 days (Tjitfosoedirdjo and Wiroatmodjo, 1984). A water hyacinth production experiment in Florida, USA reported 67 Kg fresh weight m² recovered over 13 months and 21 harvests, equivalent to 620 tons ha⁻¹ yr⁻¹ (Reddy and D'Angelo, 1990). Indeed, the great productivity of water hyacinth is responsible for both its threat as an aquatic weed and its

potential as a vegetation resource (Woomer, 1997). Nutrient rich fresh waters enhance the productivity of water hyacinth. Thus, nutritionally enriched water bodies by drainage from agricultural land, effluent discharge from industries, urban waste and inadequately treated wastewater increases the production of the weed (Woomer, 1997).

2.2.3 Habitat and distribution

The optimum growth of water hyacinth occurs in eutrophic, still or slow moving fresh water with a pH of 7, a temperature range between 28°C and 30°C, abundant nitrogen, phosphorous and potassium (Chadwick and Obeid, 1966; Knipling *et al.*, 1970; Reddy *et al.*, 1989; Ibid, 1990; Ibid, 1991). Plants will, however, tolerate a wide range of growth conditions and climatic extremes, allowing the weed to infest countries across a wide range of latitudes and climates. Good growth can continue at temperatures ranging from 22 to 35°C and plants will survive frosting, unless the rhizome is completely frozen. The seeds or seedlings can survive winter thus giving focus to sexual reproduction, seed dormancy and germination (Kunikazi, 1978; Ueki and Oki, 1979; Wright and Purcell, 1995). Plants can infest pristine, relatively low nutrient waterways and can survive for several months in low-moisture substrates. They can tolerate acidic waters but cannot survive in salt or brackish water (Penfound and Earle, 1948).

Water hyacinth often grows with other aquatic weeds including Kariba Fern, *Salvinia molesta* Mitchell, the grasses *Panicum repens* and *Paspalum distichum*, sometimes forming complex free-floating aquatic stands referred to as "floating islands" often reaching several hectares (Tjitrosoedirdjo and Wiroatmodjo, 1984; Batanouny and El-Fiky, 1984). In the Winam Gulf rotting mats of water hyacinth support successive

growth of other weeds like papyrus and hippo grass (Ochiel and Njoka 2001). This is ecological succession.

The distribution of *Eichhornia crassipes* in most regions of the World, which generally occurs between 40°N (Portugal) and 45°S (New Zealand), exemplifies the importance of temperature in its establishment. The weed's native range is in the Amazonian Brazil, with natural spread throughout Brazil and to other Central and South American countries. The spread of water hyacinth into new areas commenced in the 1880s with its deliberate introduction into the USA as an ornamental. Live plants were supposedly handed out to visitors at the 1884 New Orleans Cotton Expo. Thereafter plants continued to spread around USA and eventually around the world (Holm *et al.*, 1977; Centre, 1994; Julien *et al.*, 1996).

The earliest recorded introduction of water hyacinth to Africa was in Egypt around 1890, originally to plant in a public garden in Cairo. It escaped into the Nile Delta but did not spread beyond (Batanouny and El-Fiky, 1984). Another early introduction was to Natal, South Africa in 1910. It was also reported in the Transvaal, Southern Mozambique, Nigeria and Zimbabwe (Akinyeminjo, 1987; Gopal, 1987), probably assisted by man. The greatest spread of the weed is thought to have resulted from its introduction to the river Congo in 1942. It was established along the entire length of that river by 1956, and is believed to have crossed over into the Nile Basin through an inter-connective swampy area in Southwestern Sudan (Bebawi, 1972). This route is most likely responsible for the current biological invasion in Lake Victoria besides River Kagera (Woomer, 1997).

2.2.4 Utilisation of Water Hyacinth

Water hyacinth has been utilised by man in a variety of ways. In India the weed is used as mulch in tea estates and as a feed for ruminants and pigs (Gopal, 1987). Other suggested uses are in biogas generation, making of fibre products, in wastewater treatment and its complete combustion into "charcoal black" pigments. The potential for its economic utilization is greatly hampered by the fact that the fresh plants contain 95% water and are slow to dry and decompose (Woomer, 1997).

A unique feature of water hyacinth is its capacity to absorb high quantities of chemicals from its surrounding resulting in biological approaches to waste water treatment. Haider (1984) reported selectivity in copper, zinc and iron uptake by water hyacinth with most of these metal ions stored in the root and stem. For example, copper ion concentration (Cu²⁺) increased from 19 and 91 ppm to 131 and 1500 ppm within two days in the hyacinth stem and root respectively. Water hyacinth has also been reported to remove mercury (Lenka *et al.*, 1990), chromium (Saltabas and Akcin, 1994), cadmium (Rai *et al.*, 1995) and phenolics (Nor, 1994) from water solution.

Under favourable conditions one hectare of water hyacinth can remove 22-44 kg of Nitrogen, the same amount of Potassium, 18-34 kg of Sodium, 11-22 kg of Calcium, 8-17 kg of Phosphorous, 2-4 kg of Magnesium per day from polluted effluent. For the heavy metals, an additional 89 gm of Mercury, 104 gm of Lead, 297 gm of Nickel, 321gm of Strontium, 343 gm of Cobalt, 385 gm of Silver, 398 gm of Cadmium and 2134 gm of Phenol are removed by each hectare of water hyacinth daily (Rady, 1979). Despite the potential application of the weed to agriculture and industry, it must be borne in mind

that water hyacinth is a weed and biological invader first and a resource second (Woomer, 1997).

2.2.5 Problems associated with water hyacinth

Eichhornia crassipes is one of the most economically important aquatic plants in the world (Holm et al., 1977). The weed causes serious ecological and economic problems wherever it invades. Thick mats of the weed cause disruption to water transport, impede access to fishing areas, landing beaches and destroy fish traps and nets. It forms fertile ground for breeding of vectors of human diseases inter alia malaria, bilharzia and river blindness (Harley, 1996; Epstein, 1998). In Egypt, the bilharzia snails have been shown to prefer Potamogeton crispus followed by Eichhornia crassipes and then Panicum repens (Dawood et al., 1965).

Water bodies with a high density of this weed have a pungent smell with reduced pH and temperature coupled with an increased biological oxygen demand (BOD), chemical oxygen demand (COD), and free bicarbornate (Gopal, 1987; Frielink, 1990). Besides greatly reducing oxygen diffusion at water-air interface, the weed impedes light penetration and may completely reduce photosynthetic activity. In shallow water bodies, it shields out fauna, blocks spawning and breeding grounds for fish (Gopal, 1987). All these greatly impair the economy and health of the riparian communities.

Infestation in canals and drains causes a reduction in the flow of water below design levels thereby preventing delivery of irrigation water and drainage of fields. The National Irrigation Board's West Kano Irrigation Scheme in Kenya is a case in point. The weed also causes economic loss when it invades hydroelectric power generating

dams e.g. Owen falls in Uganda. It has been shown that evapo-transpiration through a cover of water hyacinth is always greater than evaporation from an open water surface. Hamdoun and Tigani (1977) estimated that 7 billion m³ or one-tenth of the average flow of the Nile was lost every year through evapo-transpiration by water hyacinth. This reduction in water can eventually convert open water into shallow marshes.

2.3 Control of Water Hyacinth

Several control and management strategies for water hyacinth are available. These are through legislation, mechanical removal, manual clearance, herbicides and biological control. These methods may be combined in various ways into an Integrated Control Programme including utilization (Harley *et al.*, 1996). Water hyacinth flourishes in nutrient rich fresh water such as those from agricultural land, discharge into the water from factories or urban waste and inadequately treated sewage effluent. The identification and reduction of sources of nutrient enrichment therefore becomes critical in the management of water hyacinth (Woomer, 1997).

2.3.1 Legislative Control

The regulation and control of plants and animals movement is complicated by the various modes of invasion. Some species find their way into new habitats by accident – they hitchhike in ships, planes, on traded goods or simply on travellers, while others are intentionally introduced for hunting, fishing or pest control. Others 'escape' their intended confines like the seaweed *Caulerpa taxifolia*, which was originally intended

for aquariums in Europe but escaped and is now a common sight along French and Italian coastlines (MCBI, 1998).

The International Plant Protection Convention (Kiss, 1983) whose objective is to maintain and increase international cooperation in controlling pests and diseases of plants and plant products, and in preventing their introduction and spread across national boundaries is one of the earliest examples of an attempt to limit the transboundary spread of pests and diseases. The Phytosanitary Convention for Africa, south of Sahara adopted in 1954 is another example whose objective is to prevent the introduction of diseases, insect pests and other enemies of plants into any part of Africa south of Sahara, to eradicate or control them in so far as they are present in the area and to prevent their spread. These and other multilateral treaties, conventions and protocols may help to curb the spread of noxious weeds but on careful examination, the weeds related to are those that compete with agricultural crops and not aquatic weeds like water hyacinth. There are, for example about 10 Agreements dealing with consumptive use of the waters of Lake Victoria, and those prior to World War 1 show Britain as the contracting party, but there seem to be none specifically aimed at controlling the spread of water weeds (Okidi, 1990).

In Kenya, the cultivation and transporting of water hyacinth and other invasive weeds is prohibited under the Plant Protection Act Chapter 324. That notwithstanding, there is now an urgent need to educate communities on the dangers of water hyacinth given the experience in Lakes Victoria, Naivasha and the Nairobi dam. The sale of water hyacinth plants in the streets of Nairobi has been noted with concern (Njoka, 2002). Being a fresh water weed it means that it can easily be introduced into and infest more

of our fresh water lakes and rivers. The irrigation and hydropower potential of these waters would therefore be greatly compromised. The recently formed National Environmental Management Authority (NEMA) has a duty under the law to arrest and prosecute offenders for environmental abuse. In fact, the Authority has a Public Complaints office through which the public can channel complaints without them appearing in court. The Authority has also, through an Act of Parliament revised the penalties for environmental offences.

2.3.2 Physical Control

Physical control consists of mechanical and manual removal. Baruah (1984) cites mechanical removal as offering advantage because it is rapid and physically removes the weed from the water body. Several harvesters of aquatic weeds have been developed based on bucket or rake designs (NAS, 1977). Mechanical harvesting has several disadvantages including difficulties of access by conveyors and trucks and the requirement for disposal following harvest. The greatest constraint is the initial and maintenance costs of the heavy equipment (Woomer, 1997). Nonetheless, mechanical harvesting has proven effective in developing countries such as Malaysia, where dragline excavators clear irrigation and drainage canals of heavy water hyacinth growth (Yusof, 1984). Manual removal, on its part, is difficult and may only be confined to shallow beaches for the supply of domestic water and to pave way for fishing boats. The weed has an enormous capacity for regrowth from remnant plants and seeds and therefore requires constant harvesting (Julien and Griffiths, 1999)

2.3.3 Chemical Control

Chemical treatment of aquatic weeds in general is recommended in various countries of the world. The herbicides mainly used to control water hyacinth are Diquat, 2,4-D, Ametryn, Amitrole and Glyphosate (Charudattan, 1988; Kahattab, 1988; Harley, 1994). However, it is not advisable to use chemicals in or near water bodies as this may bring about risks to the environment and human health (Pieterse, 1994). This is especially so in densely populated areas where the community draws raw water for domestic purposes from the water body as is the case of Lake Victoria. Chemical control represents the unforeseen toxic effects of residual chemicals on non-target aquatic organisms and man. The use of chemicals must be preceded by appropriate experiments, to prove their cost-effectiveness and acceptability to the local communities (Kusemiji, 1988). Herbicidal control of large infestations of water hyacinth growing under favourable conditions has rarely yielded success. This longterm commitment is often, of course, difficult to maintain and is an on-going cost (Harley, 1994). In the Sudan, environmental effects of aerially applied herbicide 2,4-D, dodecyl-tetradecyl amine salt on non-target plants are still being seen 15 years after all herbicide applications ceased (M. O. Beshir, Pers. Comm).

It is appreciable that Lake Victoria is a shared natural resource serving not only the riparian states of Kenya, Uganda and Tanzania, but also Ethiopia, Sudan and Egypt down the River Nile. Any attempts to use herbicides on the lake would pose serious threat to its non-target biodiversity besides attracting stiff economic penalties from the fish export market and environmentalists. The European Union ban on Lake Victoria fish in 1997 following fish poisoning by chemicals used for unethical fishing dealt a

big blow to the riparian economy. The use of chemicals to control the weed in the lake is therefore undesirable.

2.4 Biological Control

This is a new area of study and approach for the control of water hyacinth in Lake Victoria Basin and forms the basis of this study. The following review provides a detailed account of biological control in general and biological control of water hyacinth in specific.

2.4.1 A Historical Perspective

The concept of biological control was developed from observations by early naturalists and agriculturalists. The earliest use of predators and parastoids to control pests is lost in history, but it is known that the Chinese used the ant *Oecophylla smaragdina* to control caterpillars and large boring beetles in citrus groves in ancient times. Forskal P. recorded a similar activity, by date growers in Arabia in 1775. The first known establishment of a natural enemy moved from one country into another, was credited to de Maudave who introduced the mynah bird, from India to Mauritius to control locusts. In Europe, a predacious pentatomid bug *Picromerus bidens* was introduced against bed bugs as early as 1776. It is unclear who first correctly interpreted the phenomenon of insect parasitisation, but during the first decade of 1700s, Vallisnieri of Padua, Van Leeuwenhoek and Cestoni (a correspondent of Vallisnieri) wrote of, or pictured, parasitoids of insects (De Bach, 1974).

In Africa, Biological control has been attempted with varied levels of success. While the approach is more successful against insect pests not much work has been done on weeds especially waterweeds. The most spectacular success has been the International Institute of Tropical Agriculture (IITA) Africa-wide programme for the biological control of the cassava green mite, *Mononychellus tanajoa* and cassava mealy bug, *Phenacoccus manihoti* (Herren, 1989; Yaninek *et al.*, 1989; Neuenschwander, 1990).

A logical extension of the concept of using parasitoids and predators to control insects was the use of natural enemies to control weeds. The first major programme for biological control of a weed commenced in 1902, when fruit and flower feeding insects collected by an early entomologist, one Koebele in Mexico were introduced to Hawaii for the biological control of *Lantana camara* (Perkins and Swezey, 1924). These insects effectively checked the spread of Lantana on drier parts of the islands of Hawaii and some were later sent to Fiji, Australia, India, East Africa and South Africa (Goeden, 1978). The outstandingly successful control of the prickly pear cacti *Opuntia inermis* and *O. stricta* in Australia during the 1930s was a milestone in biological control programmes (Dodd, 1940).

Since the 1950s, there has been increased interest on biological control programmes for weeds. Julien and Griffiths (1999) have summarised the biological introductions for weed control and stated that up to 1980, there had been 174 programmes aimed at controlling 101 weed species, by organisations in more than 70 countries. The United States of America, Australia, Canada, South Africa and the Centre for Agricultural and Biosciences International (CABI) in the United Kingdom have been foremost in the search of biological control agents and the implementation of biocontrol

programmes (Harley and Forno, 1992). In Kenya, the weevil *Cyrtobagous salvinae* was successfully used to control *Salvinia molesta* in Lake Naivasha (Anon, 1998). The invasion of Lake Victoria in 1988 by water hyacinth posed the biggest challenge to biological control programmes in the East African region. By 2001 the weed had been reduced by 80% from a peak infestation of 6000 ha on the Kenyan side of the lake largely due to the *Neochetina* weevils introduced into the lake in January 1997 (Ochiel and Njoka, 2001).

A historical account of biological control would be incomplete without mentioning the enormous difficulties confronting the early explorer, in transferring colonies of living insects from their native range to the country of introduction. Usually, large heavy wooden cages containing live host plants had to be transported overland by oxcart, horse or camel, and then by ship where, as deck cargo, salt spray and searing heat took their toll. The insects were in transit for months and often underwent several generations en route, and only the healthiest and most robust species or colonies survived. Today colonies travel by air in small light packages and arrive at their destinations within a few days or hours (Harley and Forno, 1992).

2.4.2 Classical and non classical Biological Control methods

The term 'biological control' was defined by De Bach (1974) as "the study and utilisation of parasites, predators and pathogens for the regulation of host population densities". These natural enemies of the target pest are referred to as control agents. They are usually host specific and obligate feeders. Biological control is the only control method that is economical, environmentally safe and sustainable. It is environmentally friendly and, unlike chemical control, it is perfectly safe where water is used for drinking

and in fishing zones (Harley and Forno, 1992). Once biological controls takes effect, little further input is required. The agents are self-regulating and they spread to suppress new growth as it appears. The limitation to biological control is that it requires a minimum of several years, usually three to five, for the insect populations to increase to a density where the weed is in substantial decline (Harley *et al.*, 1996).

Biological control is a proven, cost-effective method for managing growth of floating aquatic weeds. Studies done on Kariba weed, *Salvinia molesta* indicate that the use of biological control agents as opposed to use of chemicals had a cost-benefit ratio of around 500:1 (Chikwenhere and Keswani, 1997). Successful programmes have been implemented against the alligator weed, *Alternanthera philoxeroides* (Coulson, 1977); *Salvinia molesta* (Room *et al.*, 1981; Anon., 1998) and water lettuce, *Pistia stratiotes*. (Harley *et al.*, 1984; Chikwenhere and Forno, 1991). Recent successes with biological control of water hyacinth, *Eichhornia crassipes*, have now been reported worldwide including East Africa (Wright, 1979; Centre, 1982; Deloach and Cordo, 1983; Cofrancesco, 1984; Goyer and Stark 1984; Beshir *et al.*, 1984; Irving and Beshir, 1984; Jayanth, 1988; Cilliers, 1991; Chikwenhere, 1994; Kannan and Kathiresan, 1999; Ogwang' and Molo, 1997; Mallya, 1999; Ochiel *et al.*, 1999).

Biological control may be divided into classical and non-classical control. The introduction of control agents into a region that is not part of their natural range, to suppress the populations of target weeds exotic to the area is called 'classical biological control'. The control agents are usually arthropods or plant pathogens and other natural enemies like nematodes (Waage and Greathead, 1988). It results into the establishment of the control agents equilibrium with the target weed thus no longer causing economic /

environmental injury. Classical biological control does not pollute the environment. The control agents are chosen for their host specificity. This method of weed control is entirely compatible with responsible environmental management (Hokkanen, 1985; Harley and Forno, 1992).

Whereas classical biological control is self-perpetuating and self-regulating and a control agent becomes a permanent part of biota in the region where it is established, in 'non classical biological control', the agent is not part of the biota, or, if it is, it occurs only at a population density which does not exercise acceptable control of the weed (Waage and Greathead, 1988).

In non-classical biological control, fungal plant pathogens are applied to the target weed in inundative or augmentative doses. The preparation containing the fungal pathogen is known as 'mycoherbicide'. Mycoherbicides may give comparable, or perhaps better control than chemical herbicides but their special attributes are a high level of specificity and reduction in the amount of pesticides being added to the environment. Mycoherbicides are particularly well suited to controlling weeds in annual crops (Harley and Forno, 1992). However, optimisation of strategies for the use of mycoherbicides requires further study of the basic factors involved in fungal-weed disease process in production, application and in compatibility with other pesticides (Greaves and Macqueen, 1990; Van Dyke, 1990),

2.5 Biological control of Water Hyacinth

2.5.1 Natural enemies and their population

"Biological control" is the use of host specific natural enemies to reduce the population density of a pest. This is acclaimed to be the safest, environmental friendly and most sustainable method of control. Natural enemies of water hyacinth are known to occur in its area of origin. These include the two weevils of the species *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Curculionidae: Coleoptera), the moths *Niphograpta albiguttalis* Warren, *Xubida infusellus* Walker (Lepidoptera: pyralidae) and an oribatid mite *Orthogalumna terebrantis* Wallwork (Acarinae). Another new natural enemy is the Hemipteran bug *Eccritotarsus catarensis* (De Loach and Cordo, 1978; Julien *et al.*, 1996). These natural enemies have continued to control the weed in its native range in the Amazonian Brazil (Centre *et al.*, 1988).

Water hyacinth *E. crassipes* is a native of the Amazonian Brazil, South America where it is known to have a number of natural enemies. These agents keep the weed in check thus not reaching the economic / environmental injury level. The changes in the population density of a weed before and after the establishment of biological control agents are graphically illustrated in Figure 2.1.

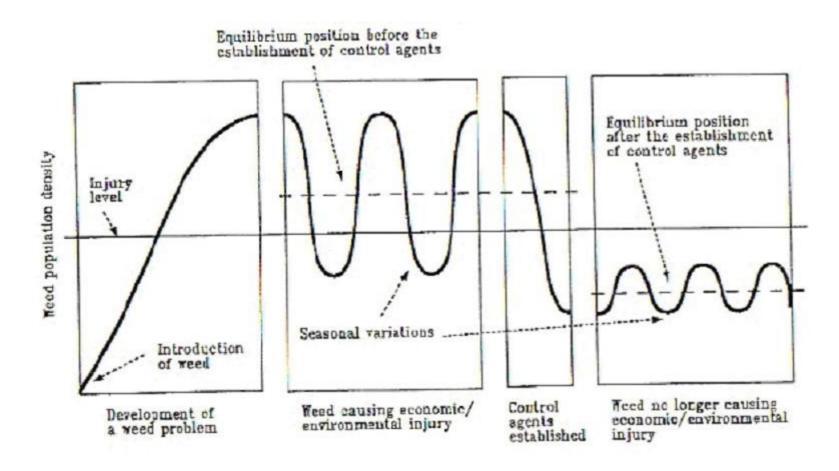


Figure 2. 1: Diagramatic representation of the changes in the population density of a weed before and after the establishment of biological control agents.

(After a training booklet sponsored by UNDP/FAO in cooperation with IITA/Biological Control Program).

In East Africa where the weed was recently introduced, there are no known existing natural enemies for this aquatic weed. The weed therefore continues to proliferate without check. The fertility of the lake waters due to pollution and ideal climatic conditions adds to the gravity of the problem. The attempts to control water hyacinth in East Africa using the imported natural enemies of the genus *Neochetina* are a case of classical biological control.

2.5.2 Previous Biological control attempts

The United States Department of Agriculture (USDA) initiated biological control of water hyacinth in 1961 (Harley and Forno, 1992). Subsequently, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), International Institute of Biological Control (IIBC), Plant Protection Research Institute (PPRI) and USDA have carried out surveys for natural enemies in the native range of the weed in South America. The main biological control agents used successfully against the weed are the two exotic weevil species, *N. eichhorniae* and *N. bruchi*, the lepidopteran moths, *Niphograpta albiguttalis* Warren and *Xubida infusellus* Walker and an oribatid mite, *Orthogalumuna terebrantis* Wallwork.

Of these, the exclusively host-specific weevils *N.eichhorniae* and *N. bruchi* are the most successful and important biological control agents used against the weed with notable success in Argentina, Australia, India, Sudan, USA and South Africa (Oso, 1988; Harley, 1990; Julien, 1992; Centre, 1994; Cilliers, 1991).

Some phytopathogenic fungi have also been reported to attack water hyacinth. The characteristics that make them desirable biological control agents are that they are easily cultured, disseminated and once established may not require constant reapplication. They

also do not affect man, his animals, wildlife or fish (Harley and Forno, 1992). The fungus *Cercospora rodmani* has been found to effectively control water hyacinth at Rodman Reservoir in Florida, USA (Conway *et al.*, 1978). *C. rodmani* is host specific to water hyacinth and can spread from infected areas causing large areas of mat to die and sink (Conway and Zettler, 1971). It has also been formulated as a mycoherbicide. Other fungi like *Acremonium zonatum*, *Drechslera specifera*, *Fusarium equisetti*, *Alternaria alternata* and *Phoma sorghina* have also been evaluated and found to have a potential for control of water hyacinth (Evans, 1987). While many plant pathogens exert an appreciable limiting pressure on target water hyacinth populations, they seldom eliminate it (Freeman *et al.*, 1974).

2.6 Neochetina weevils as biological control agents of water hyacinth

2.6.1 Taxonomy and biology of *Neochetina* weevils

The genus *Neochetina* is comprised of six species whose native range is primarily South and Central America. They are classified in the largest insect order Coleoptera and in the family Curculionidae. This is currently the largest family of animals in the world with at least 3,600 genera and approximately 41,000 species, generally referred to as weevils. The Curculionidae is probably the most economically important family of Coleoptera and as important as the Noctuidae of the Lepidoptera (Booth *et al.*, 1990). Other notable species of this family include the oil palm weevil, *Elaeidobius kamerunicus* (Faust) which was introduced from West Africa to Malaysia in 1982 to pollinate oil palms, the Salvinia weevil, *Cyrtobagous salvinae* which was successfully used to control *Salvinia molesta* in Lake Naivasha, Kenya and the notorious American cotton boll weevil *Anthonomus grandis* which

prevents the development of flowers by its feeding thus causing enormous crop losses in cotton yield in the USA.

The Neochetina weevils are all are semi-aquatic, covered with a layer of very dense water repellent scales and feed only on species of plants in the family Pontederiaceae (O'Brien, 1976). The adults of Neochetina bruchi and N. eichhorniae can usually be distinguished by the colour and pattern of the scales covering the elytra. N. bruchi ranges in colour from uniform tan or brown with no distinct markings to brown with a broad crescent-shaped or chevron-like tan band across the elytra. N. eichhorniae never has the tan band and is usually grey mottled with brown. The colour pattern is associated with scales and specimens may be difficult to identify if the scales are missing or the specimens are dirty or wet. Both species have two shiny dark lines on the elytra on either side of the mid-line. This short line is actually a tubercle or ridge and its position varies between the two species. On N. bruchi the tubercles are situated very near mid-length. Although the position of the tubercle is more variable on N. eichhorniae, they are usually situated further, in front of mid-length. A more subtle character separating the two species concerns the lines (striae), which run lengthwise and nearly parallel to one another on the elytra. These striae are actually grooves. On N. bruchi (Plate 2.1) the striae are relatively fine and shorter whereas on N. eichhorniae, they are relatively coarse and longer (Plate 2.2). This confers an overall smoother textual appearance on *N. bruchi* than *N. eichhorniae* (Warner, 1970).



Plate 2. 1 Adult Neochetina bruchi, natural enemy of water hyacinth



Plate 2. 2: Adult Neochetina eichhorniae, natural enemy of water hyacinth

The biology and life cycle of these weevils has variously been described by Warner (1970); Deloach and Cordo (1976a); Ibid (1976b); O'Brien (1976) and Center *et al.*, (1988). Both have similar feeding habits, but some aspects of their biology differ (Harley, 1990). They co-exist successfully owing to differences in ovipositional behaviour and seasonal abundance (Jayanth, 1988). The eggs, larvae, and pupae of both species are very similar and virtually indistinguishable from one another.

The eggs (Plate 2.3) are whitish, ovoid and about 0.75mm in length. Since they are embedded in the plant tissue, they can usually only be found by dissecting the plant. Eggs of both species are deposited directly in the plant tissue. The female bores a hole into the lamina or petiole into which it lays eggs. *N. eichhorniae* deposits only one egg per hole whereas *N. bruchi* deposits several. Either species may also place eggs around the edge of the adult feeding pits. DeLoach and Cordo (1976a) reported that *N. bruchi* prefers to oviposit in leaves with inflated petioles (bulbous) especially those at the periphery of the plant, while *N. eichhorniae* preferred the tender central leaves or the ensheathing stipules at the leaf bases. The eggs hatch within 7 - 10 days at 24° C (Center *et al.*, 1988).

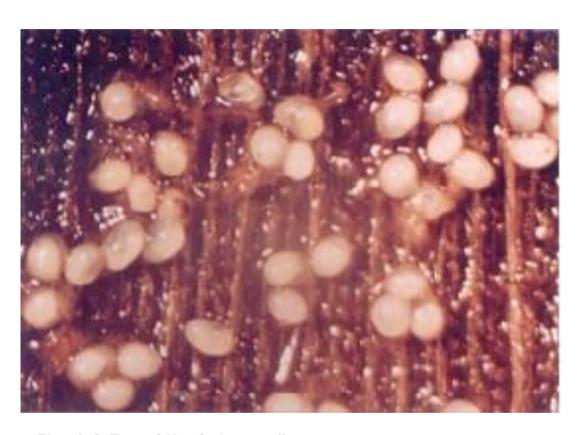


Plate 2. 3: Eggs of *Neochetina* weevils

The larvae are white or cream coloured with a yellow-orange head (Plate 2.4). The first instar larvae that are very small (head diameter of 0.3mm) burrow under the epidermis and work their way towards the base of the leaf. They pass through 3 larval instars. The first moult occurs when the larvae are about 10 days old and the second at about 2 weeks later. As they grow larger the galleries or feeding burrows become larger. Third instars are generally located at the petiole bases and may enter the stem (rhizome) and excavate small pockets near the point of insertion of the leaf. They occasionally burrow up the stem to enter the base of younger petioles and sometimes reach the stem apex and destroy the apical bud. The larval period requires 30 - 45 days with *N. bruchi* developing somewhat faster than *N. eichhorniae* (Centre *et al.*, 1988). Appreciably, the larval stage is the most destructive and by destroying the apical bud, effectively kills the plant.



Plate 2. 4 Larval stage of *Neochetina* weevils

The fully developed larvae burrow out of the stem and move to the upper root zone just under the surface of the water. They cut off small lateral rootlets and form a cocoon around themselves. This cocoon is attached to one of the roots. The larva now moults a third time to become a pupa (Plate 2.5). This is an inactive stage occurring during the transition from larva to adult. It is not known with certainty how long this stage lasts but previous estimates indicate about 7 to 10 days (Warner, 1970).

The adults emerge by splitting the cocoon and climbing onto the emergent leaves of the plant to feed and mate. The female weevils begin to lay eggs within a few days after emerging from the pupa and most are deposited within the first week. A single female *N. bruchi* will deposit up to 300 eggs while a female *N. eichhorniae* can deposit in excess of 400 eggs during her lifetime (Centre *et al.*, 1988). About 90% of the eggs are deposited within a month after the female emerges although the adults may live for over 9 months (DeLoach and Cordo, 1976a).

2.6.2 Status of Biological control using *Neochetina* Weevils

The use of biological control agents in various parts of the world has had various levels of success. In the USA, large tracts of the weed were brought under control using *Neochetina* weevils in Florida (Centre, 1994). In the Indian sub-continent, 3 weevils per plant were shown to provide successful control in Lake Veeranum in the Tamil Nadu State of South India (Jayanath, 1988). In Africa successful control of water hyacinth has been reported in Egypt, Sudan, South Africa, Zimbabwe and Uganda (Beshir *et al.*, 1984; Cilliers, 1991); Chikwenhere, 1994; Ongwang' and Molo, 1997; Mallya, 1999).



Plate 2. 5: Pupal stage of *Neochetina* weevils

2.6.3 Attributes of using *Neochetina* Weevils against water hyacinth

These weevils are reported to be obligate feeders on water hyacinth. This is a very important attribute in that the weevils would starve to death if not fed on water hyacinth, as they would not feed on any other plants. Further the weevils cannot complete their life cycle on other plants except water hyacinth. Of particular significance is the pupa stage of the weevil, which occurs in the root hairs of the host plant. This also means that the weevils cannot complete their life cycle in terrestrial plants.

The weevils reproduce fast in a suitable climate and can therefore cope with a resurgence of the weed. The long larva period ensures maximum damage to the petioles while the feeding scars inflicted by the adult provide opportunity for pathogenic fungi.

2.7 Description of the study area

2.7.1 Location and size

Lake Victoria fills a basin depression in the centre of the Great African Plateau located between the Eastern and Western flanks of the Great Rift Valley. The shores of the lake are variable but typified by indentations to the East, deep inlets to the South, papyrus as well as swamps to the West and a flat, indented and forested coast to the North (Graham, 1929). The Lake is located between latitude 0°20'North to 3°South and longitude31° East to 34°52' East. It has a total area of 69,000 km² shared between Kenya (6%), Uganda (43%) and Tanzania (51%). The land along the Kenyan

shoreline is approximately 157,000 ha of which 77,600ha are in the Southern shores while 79,400 ha are in the Northern shores of the lake (Graham, 1929).

The Kenyan portion of Lake Victoria commonly known as Winam Gulf, Nyanza or Kavirondo covers an area of about 3,800km² and extends from Mbita in Suba district to the regionally strategic port of Kisumu and on to Busia in the North. Nine administrative riparian districts namely Suba, Migori, Homa Bay, Rachuonyo, Nyando, Kisumu, Bondo and Siaya all in Nyanza province and Busia in Western Province share the Gulf. Inclusive of the water surface these districts cover an area totaling about 10,880 Km². The lowest points in altitude are at 1,100m while the highest is at 1,430 m. (Graham, 1929). The immediate hinterland of the lake is endowed with numerous satellite water bodies of different sizes all of which are currently infested with water hyacinth (Fig 2.1).

2.7.2 Climate

The lake lies across the equator and thus experiences an equatorial climate throughout the year. It plays a significant role in the climatology of the surrounding areas. The lake has a strong local circulation, which is due to the lake and land breeze circulations associated with the differential heating and cooling of water and land surfaces.

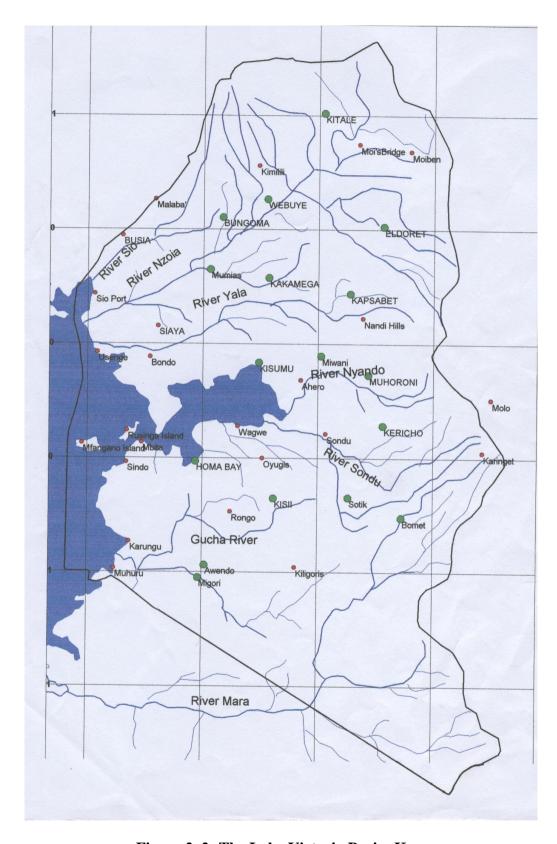


Figure 2. 2: The Lake Victoria Basin, Kenya

The strong temperature and pressure gradients induce a breeze from the lake to the land surface during the day and from the land to the lake at night. This day and night circulation induces low / high pressure inside the lake during night / day respectively and is locally known as the Lake Victoria trough (Okeyo, 1986).

The rainfall climatology of this area has variously been described by Lumb (1970) and Asnani and Kinuthia (1979) among others. According to Lumb (1970) most areas to the east of the lake receive their share of rainfall and thunderstorms during the afternoon hours. This is attributed to the lake breeze, which gets counteracted by the easterlies in the lower troposphere and creates a convergence zone over the highlands. During the night, a low-pressure zone dominates over the lake leading to the rising of the moist air. This moist air then forms clouds, which drift westwards bringing rainfall to the western parts of the lake during the early morning hours.

The temporal distribution of rainfall around the lake is governed by the Intertropical Convergence Zone (ITCZ), the Westerly Congo Air Mass (CAM), local mesoscale features and large scale zonal circulation patterns (WMO, 1982). Mungai, (1984) describes two rainfall peaks centered around March-May and October-November in the lake region. These peaks are associated with the ITCZ, which crosses the lake twice a year when most of East Africa is under the influence of the northeast and southeast-southwest winds. The July / August peak is associated with the westerly influence of moist currents from the Congo / Zaire basins (Lumb, 1970).

The distribution of the rainfall in the region is bimodal. The mean annual rainfall over the lake catchment varies from more than 2,000 mm along the western edge to less than 700

mm near the eastern shores while evaporation from the lake surface ranges from 2,000 to 2,200 mm per annum resulting in an annual rainfall deficit (Crul, 1994).

The lake breeze circulation modifies daytime temperatures around the lakeshores. Consequently, on the eastern shores the maximum daily average temperature varies between 29°C-30°C while on the western and northern shores, it is about 26°C. During the night, the minimum temperatures average between 16°C and 17°C in the north and west while to the south and eastern parts, the corresponding figures are 17°C-18°C. (WMO, 1982).

2.7.3 Geophysiography

The Lake Victoria Basin is one of the five major drainage basins in Kenya. Other major drainage basins in Kenya include the Rift Valley, Rivers Tana, Athi and Ewaso Ng'iro (LBDA, 1987). The Basin has a mean runoff of 149 mm, an evaporation of 1,096 mm, a mean rainfall of 1,245 mm per annum and a temperature range of between 20 - 24°C (Heyer et al., 1976; Corbett et al., 1999). The lake's major outlet is the Nile while its largest single affluent is River Kagera from the Rwandan highlands, which accounts for about 40% of the total annual inflow. Most of the remaining surface inflow comes from rivers originating in the western highlands of Kenya, which account for 35-44% of the total inflow. Most of these rivers pass through natural woodlands, agricultural and industrial zones, rendering them more susceptible to eutrophication and pollution (Madati et al., 1982; Okidi, 1994; Afullo, 1995). The main rivers draining the Kenyan Basin of Lake Victoria include Yala, Nzoia, Nyando, Sio, Kuja-Migori, Sondu-Miriu and Kibos.

The soils occurring on the lakeshore shows complicated distribution patterns. In general, the soil is colluvial, originated from igneous rocks forming hilly lands. They are characterised by dark yellowish to brownish colour, good drainage and high gravel contents. Besides, the black alluvial soil is locally identified along the lakeshore. It is quite similar to the soil covering Kano plains, imperfectly drained, deep and fertile. The origins of these types of soils are partly the lacustrine deposits, but mainly the river alluvial deposits transported by small rivers, which incise the lakeshore and eventually flow into the lake (LBDA, 1987).

The Lake Basin region is divided into 9 agro-ecological zones (Anon, 1989) as follows:

- i) UM 1- Tea / Coffee zone
- ii) UM 2- Coffee zone
- iii) UM-3 Marginal coffee zone
- iv) UM 4 Sunflower, maize / upper sisal zone
- v) UM 5- Lower Midland zone
- vi) LM Lower Midland zone
- vii) LM 1- Sugarcane zone
- viii) LM 2 Marginal sugarcane zone
- ix) LM 3 -Cotton zone
- x) LM 4 Marginal cotton / sisal zone
- xi) LM 5- Livestock / millet / zone and marginal sisal zone.

These zones can further be classified into two distinctive ecological zones – the high rainfall zone and the savannah zone along the lakeshore (Jansen, 1973). The lakeshore savannah zone coincides with the lakeshore area and much of the hinterland for distances of about 20 km on either side of the Winam Gulf. This zone has lower and less reliable

rainfall. Generally, the rainfall pattern in the lakeshore region permits only one successful crop in a year whereas in the high rainfall zone two cropping seasons are widely practised (Jansen, 1973).

2.7.4 Demography

The area under study is highly populated with a total population of about 3 million (close to 11% of Kenya's human population) at a density of 290 persons / Km² (Anon, 1999). The population distribution per district is shown in Table 2.1. Migori district in the former South Nyanza has the largest land area of 2005km² and also the highest population of 514,897 persons. Kisumu district has the least area of 919km² but has a high population of 504,359 and the highest density at 549 people/km². This is expected as the district hosts the large urban city of Kisumu, a regional economic hub.

These riparian districts have a large population, which depends on the lake for fishing, transport, domestic, agricultural and industrial water supply. The invasion of the water hyacinth therefore greatly affects their livelihood. The weed is also known to provide good breeding grounds for disease vectors thus posing serious threat to community health.

Table 2. 1: Population distribution in the Winam Gulf Districts

| District | Area (Km2) | Population | Density / Km ² |
|-----------|------------|------------|---------------------------|
| Bondo | 987 | 238,780 | 242 |
| Busia | 1,124 | 370,608 | 330 |
| Homa Bay | 1,160 | 288,540 | 249 |
| Kisumu | 919 | 504,359 | 549 |
| Migori | 2005 | 514,897 | 257 |
| Nyando | 1,168 | 299,930 | 257 |
| Rachuonyo | 945 | 307,126 | 325 |
| Siaya | 1,520 | 480,184 | 316 |
| Suba | 1,055 | 155,666 | 147 |
| Total | 10,883 | 3,160,090 | 290 |

Source: 1999 Population and Housing Census, Republic of Kenya.

2.7.5 Economic Activities

The main economic activities in the Gulf region are agriculture and fishing. The land tenure system is still largely under the traditional communal system. The people are basically small-scale farmers who practice mixed farming with low-level farm inputs. The subsistence farming includes maize, beans, sorghum, groundnuts, simsim, and livestock. Most agricultural activities in the area are at subsistence level, with cotton, sugar and rice as the cash crops (Ogutu, 1988). These sectors have lately collapsed with the closure of all cotton ginneries, Miwani sugar factory and the rice mills at Ahero. They do not therefore serve as an alternative to fishing (Ikiara, 1999).

Fishing in the Gulf has for a long time been a major source of protein and income. Fishing is therefore the most important economic activity for most of the year except for periods coinciding with planting, weeding and harvesting when demand for agricultural labour is high. Due to the economic pressure, the majority of the fishermen are now permanent (Ogutu, 1988).

The invasion of the lake by water hyacinth has greatly hampered this sector of the economy by clogging fish landing sites and impeding access to fishing sites besides stagnating lake transport, which facilitates trade and commerce across the riparian states. It is now more labour intensive to land fish than before the invasion of water hyacinth. Paradoxically, there are unsubstantiated reports linking water hyacinth to the emergence of traditional fish species that had long disappeared from the catches.

Wood fuel for drying fish exerts pressure on the available trees while overgrazing enhances soil erosion. The area is also prone to serious flooding during the long rains.

The agricultural potential cannot be fully realised and result in high poverty levels. These are serious environmental issues in the study area.

The more enterprising people have been making products from water hyacinth for sale. These items include chairs, cards, boards, ropes and crafts. The Kisumu Innovation Centre (KICK) has been particularly instrumental in this regard. A self-help women centre in Homa Bay has also been involved in utilising hyacinth for commercial gain. Some farmers, near the lake have also been harvesting the weed for mulching. While these efforts are to be complemented, they are not sustainable as the production costs are uneconomical. Water hyacinth is over 90% water and therefore large quantities of the weed are required to make a unit of these products. The weed is therefore of negative economic importance (Woomer, 1997).

CHAPTER THREE

3.0 General Materials and Methods

3.1 Acquisition of *Neochetina* weevils

The adult weevils used for these studies were imported by Kenya Agricultural Research Institute (KARI) from the International Institute of Tropical Agriculture (IITA) in Benin and the Plant Protection Research Institute (PPRI) in South Africa. Some weevils were also obtained from the National Agricultural Research Organisation (NARO) in Uganda. It is appreciable that all these weevils were originally from their native range in the Amazonian Brazil.

3.1.1 Quarantine

The weevils were then subjected to quarantine and host specificity tests at KARI's National Agricultural Research Centre (NARC), Muguga as required by the Kenya Plant Health and Inspection Services (KEPHIS). The weevils received from the various sources were transferred onto water hyacinth plants collected from Lake Naivasha. The plants with weevils were then placed in plastic containers measuring 25 x 25 x 50 cm. The containers were covered with muslin cloth and put in a Controlled Temperature (CT) room maintained at 27°C and 70% Relative Humidity under a 12 hour Dark: 12 Light regime. The light source was a fluorescent 240v, 110w bulb operated by a thermostat. Test insects for the host specificity tests were obtained from these cultures. The necessary quarantine procedures were followed to ensure the weevils and the water hyacinth plants used on transit did not escape from the quarantined precinct. Both weevil species were investigated for host specificity, as

part of the quarantine requirements, before they were released to Kibos for these and other studies (Mambiri *et al.*, 1994).

3.2 Selection and collection of water hyacinth plants

Whole, fresh and healthy water hyacinth plants were selected and pulled from the Small Water Bodies along the Kisumu-Kericho highway, loaded into a trailer hitched on a tractor and transported to the National Fibre Research Centre, Kibos. The plants were then put into five Galvanised Corrugated Iron (GCI) holding tanks measuring 148cm x 94cm, with a 2000 litre capacity. The tanks were painted on the inside with gloss paint to avoid corrosion. Water was then added and the plants introduced. To enhance plant growth, 50 grams of NPK fertiliser were applied in the tank every month. These healthy plants were then used as a nursery for the weevils.

3.3 Rearing and maintenance of the weevils

The site for the rearing and maintenance of the weevils was located at KARI's National Fibre Research Centre (NFRC), Kibos. The Centre lies at $00^0\,03'\,58.2''\,S$ and $034^0\,48'\,46.4''E$ at an elevation of 1183 metres. Its close proximity to the lake (8km) makes it ideal for this exercise. Within the Centre, an isolated area measuring 10 m x 5 m was cleared and fenced. The floor was compacted and then lined with $\frac{1}{2}$ inch stone ballast to avoid muddy contamination. The floor was thus kept relatively weed free.

Six large polythene tanks with a capacity of 3,300 litres were used for the rearing of the weevils. The tanks were arranged at 1 metre apart within the laboratory space. Before

introducing the weevils, the tanks were filled ³/₄ way with water and selected healthy water hyacinth plants placed in each tank (Plate 3.1).

Before introduction into the rearing tanks the weevils were separated into the two species and sexed using the characteristics described by Julien *et al.*, (1999). Using a magnifying hand lens the weevils were also screened for physical deformities to ensure only the healthy individuals were selected for mass rearing. Some 100 adult weevils of both sexes were put on the plants in the tanks. The two species were reared in separate tanks. Each tank was clearly marked with the name of the species and the date of introduction. The newly hatched weevils were harvested and used in these studies.

3.4 Selection of field study sites

Due to the migratory nature of water hyacinth in the open lake, it was difficult to investigate the lake-wide occurrence and distribution of the weevils. These studies were therefore conducted in some selected hidden beach sites and small water bodies (SWB) in the Lake Victoria Basin. A preliminary survey was conducted in the study area to identify suitable sites (Plate 3.2). These sites were selected on the basis of the occurrence of resident water hyacinth mats. The sites were also expected to have sufficient water throughout the year to allow long-term study. Three sites were thus identified each in the riparian districts of Busia, Kisumu and Nyando.



Plate 3. 1: Neochetina weevils rearing tank



Plate 3. 2: Author (right) and a colleague sampling in a selected site

3.4.1 Mubwohola swamp, Budalangi (Busia district)

This site is about 10 km from Port Victoria, a busy lakeside town in Busia district, Western Province. The SWB was identified during the preliminary survey of these studies. The site's Global Positioning System (GPS) location is 00°07'64. 6" N and 034°00'31. 8"E. The site is at an elevation of 1173 metres a.s.l and initially covered an area of 6300 m². This site, however dried up due to drought, and data was taken for only six months instead of the planned 12 months.

3.4.2 Okana swamp, Kisumu district

This site lies at about 22 km from the port of Kisumu on the Kisumu-Kericho highway. Its location by GPS is 00°09'07. 3"S and 034°51'24. 5".E. The site has an elevation of 1284 metres and covered an area of 682 m². There was enough water at this site and data was taken consistently every month for 12 months.

3.4.3 Otho swamp, Nyando district

This small water body lies at about 42km from the shores of Lake Victoria on the Kisumu-Kericho highway. Its location by GPS is $00^0 \, 08' \, 50.6$ "S and $035^0 \, 00' \, 12.1$ " E. It is located at an altitude of 1308 metres and covers an area of 793 m². Data was consistently taken at this site for 12 months, as there was no desiccation.

3.5 Data handling and analysis

The data from the various experiments were compiled in appropriate data sheets and analysed separately. The count data were transformed using the log transformation. For the life cycle and fecundity studies an analysis of variance ANOVA was done

followed by t-test to compare the characteristics of the two weevil species. For the survivorship and life table studies, the data was analysed using the General Linear Model run on the Minitab Statistical Package Version 13.1 (Minitab Inc., 2000).

To calculate the weevils' survival rate, the Ricker (1975) formula was used:

$$N_2 = N_1 e^{-mt}$$

Where,

 N_1 = Numbers reaching stage i

 N_2 = Numbers reaching stage i+1

m = Instantaneous rate of decrease in numbers

t = Time interval as a fraction of the year

e = Natural logarithm, (2.71828)

To determine the weevil impact on water hyacinth, data was recorded on the number of ramettes, number of petioles, number of damaged petioles, petiole length, laminar area and the number of feeding scars. The number of adult weevils by species was also recorded. The data was taken over a period of 12 months at Okana and Otho while at Budalangi it was taken for six months. The data was analysed using the General Linear Model (GLM) on Minitab Statistical Software.

CHAPTER FOUR

4.0 Life cycle and Fecundity of *Neochetina* weevils

4.1 Introduction

Life cycle studies are essential for the successful implementation of biological control programmes. The number of days taken from egg to adult stage is referred to as "generation time" and varies from species to species and under different conditions. Julien et al., (1999) have developed a generalised life cycle of the *Neochetina* weevils (Figure 4.1). The longevity of the destructive stage is particularly important and in the case of the *Neochetina* weevils, it is the larva. A longer larval stage causes more damage on the petiole thus resulting in quicker death of the plant. On the other hand, insects with a shorter generation time produce higher numbers of the destructive stage from several generations in a year than the insects with long generation time. This, coupled with the egg laying capacity (fecundity), is an important factor in the choice of a biological control agent (Harley, 1990).

"Fecundity" is a measure of the total egg production of an insect while "fertility" is the number of viable eggs laid by a female; the former is often easier to measure. In those insects where all the eggs are mature on hatching the fecundity may be estimated by examining the ovaries as Davidson (1956) did with sub-imagines of a mayfly, *Cloeon spp*. But more often eggs mature throughout much of adult life and fecundity is measured directly by keeping females caged under natural conditions and recording the total number of eggs laid (Huffacker and Spitzer, 1950; Spiller, 1964; Taylor, 1975; Hard, 1976). The latter is the case with the insect species under study.

Oviposition behaviour varies considerably with different insect species. In some species, oviposition occurs in or near the suitable larval food. But in others, gravid females simply drop the eggs at random while flying low or feeding (Ross, 1965). The former case is common for many parasitic or predatory species as described by Richards and Davies (1977). Understanding the oviposition behaviour of *Neochetina* weevils on water hyacinth before releasing them in a new environment (Lake Victoria) for biological control of water hyacinth is important for ensuring survival and effectiveness of the operation. Similarly, it is important to understand the lifecycle, longevity and fecundity of the phytophage to ensure success of the control programme.

Like in other insects, oviposition, fecundity and life cycle of phytophages such as *Neochetina* weevils, are greatly influenced by several factors. Within a given population, the rate of oviposition will be influenced by nutrition, temperature and other climatic factors (Southwood, 1978). The influence of these factors can be studied in the laboratory and incorporated into a regression equation to estimate its effect on wild populations. The observations of Richards and Waloff (1954) show that it may be justified to extrapolate data from the laboratory to field populations, but detailed ecological and population biology studies are necessary to ensure establishment and effectiveness of biological control agents.

Eggs of both species of *Neochetina* are deposited directly in the plant tissue. The female chews a hole into the lamina or petiole in which to lay eggs. *N. eichhorniae* deposits one egg per hole whereas *N. bruchi* deposits several. Either species may also

place eggs around the edge of the adult feeding pits (Center *et al.*, 1988). DeLoach and Cordo (1976a) working in Argentina reported that *N. bruchi* preferred to oviposit in leaves with inflated petioles, especially those at the periphery of the plant while *N. eichhorniae* preferred the tender central leaves or the ensheathing stipules at the leaf bases. A single female *N. bruchi* will deposit up to 300 eggs and a female *N. eichhorniae* can deposit in excess of 400 eggs during her lifetime. They further observed that about 90% of the eggs are deposited within a month after the female emerges although the adults may live over nine months. The two weevil species were imported for the first time ever in Winam Gulf, Lake Victoria, Kenya to control a new invasion of water hyacinth. Detailed biological data was therefore needed to ensure effective control of the weed in the Winam Gulf conditions.

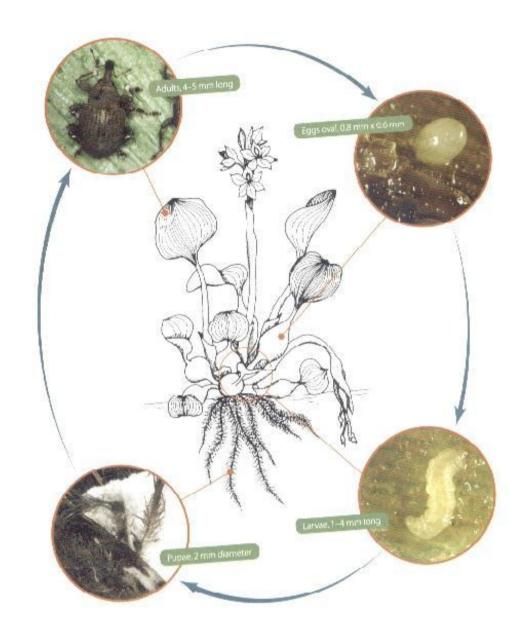


Plate 4. 1 Generalised life cycle of the *Neochetina* weevils. (Adopted from Julien *et al.*, 1999)

4.2 Objectives

4.2.1 Overall objective

To determine the life cycle and fecundity of *Neochetina* weevils to enhance their successful use in water hyacinth control in the Lake Victoria basin.

4.2.2 Specific objectives

- To determine and compare the fecundity of the two *Neochetina* species under semi controlled laboratory conditions.
- 2. To determine and compare the life cycle and development of the two *Neochetina* species under semi controlled laboratory conditions.

4.2.3 Hypotheses

- 1. The fecundity and oviposition behaviour of the two *Neochetina* species are not significantly different.
- 2. The life cycle and development durations of the two *Neochetina* species are not significantly different

4.3 Materials and Methods

4.3.1 Fecundity of *Neochetina* weevils

For each species, one mating pair of freshly emerged weevils was placed in each of ten 500ml plastic containers. Three fresh water hyacinth leaves and a 5cm long bulbous petiole cut diagonally on both ends were provided for feeding and egg laying respectively. A small amount of water (100ml) was provided to avoid desiccation. The containers were then secured on a wooden bench in the open-air laboratory at Kibos.

The adults were allowed to mate and lay eggs on the petioles for as long as they survived. Egg count was done every two days and recorded for each container while fresh petioles and leaves were provided after every count. To record the number of eggs laid by each female weevil, a disc of about 2 mm was sliced off from both ends of the petiole and observed under a hand lens. The stump petiole was also observed. This exercise was continued until either no more eggs could be recovered or the female weevil died. Each of the 10 pairs placed in 500ml plastic containers formed a replicate giving a total of 10 replications.

Data was also taken on daily temperature using a maximum and minimum thermometer while the relative humidity was recorded using a wet and dry bulb thermometer. The oviposition rate was determined by counting the number of eggs laid by each female per day. Fecundity was obtained by counting all the eggs laid by each female throughout its lifetime. The duration of egg laying was found by counting the number of days from the onset to the end of oviposition.

4.3.2 Life cycle and development of *Neochetina* weevils

To determine the life cycle and development of the two species, *Neochetina bruchi* and *N. eichhorniae*, twenty sexed pairs of each species were harvested from the respective mass rearing tanks. Each pair was placed in 500 ml plastic container and provided with three water hyacinth leaves and a 5cm long bulbous petiole for feeding

and egg laying respectively. To avoid desiccation, 100 ml of water was added to each container. The containers were covered with muslin cloth to keep off other insects. The containers were then placed on a wooden bench in the open-air laboratory at Kibos.

This set up was used to study the life cycle and development of the two species using standard statistical techniques as follows:

4.3.2.1 Egg to larva duration

Two sets of nine 500 ml plastic containers each for *Neochetina bruchi* and *N. eichhorniae* were arranged in a Complete Randomised Design replicated three times in the laboratory to investigate the egg to larva duration. In each container, sets of 10 eggs laid on 2mm water hyacinth petiole discs harvested from the rearing tanks were introduced. A little amount of water (100 ml) was added to avoid desiccation. The sets were checked daily for hatching until no further hatching was observed. The number of eggs hatched gave fertility data. The duration (days) from egg to larva gave the period of incubation.

4.3.2.2 Larva to pupa duration

To study the developmental period from larva to pupa of *Neochetina bruchi* and *N. eichhorniae*, two sets of nine 20 litre plastic buckets were filled up to ³/₄ level with water. One healthy water hyacinth plant was then placed in each bucket. Sets of ten eggs borne on 2mm petiole discs were harvested from the respective species rearing

tanks. The discs were then inserted at mid length of the plant's petioles using sterile scalpels to make the incision. Each bucket was then covered with an insect proof muslin cloth mounted on a 75 cm x 75 cm wooden cage (Plate 5.1). The duration (days) from the first instar larvae to pupal formation was recorded to give the larval development period.

The experiment was conducted in an open-air laboratory at NFRC Kibos. It was set up in a Complete Randomised Design and replicated three times.

4.3.2.3 Pupa to adult duration

It is noteworthy that the pupa stage of these weevils occurs in the submerged roots of the plant. To study the developmental period from pupa to adult for the two weevil species, two sets of nine 500ml plastic containers were arranged in a complete Random Design and replicated three times at NFRC Kibos. In each container, 10 freshly formed pupae borne on root hairs were introduced. The containers were then covered with an insect proof muslin cloth. The duration (days) taken from pupa to adult emergence was recorded.

4.4 Results

4.4.1 Fecundity

A two-way analysis of variance showed that there were significant (P<0.05) differences between the egg laying capacities of the two weevil species and also between the weeks.

After removing the effect of time in weeks, re-analysis of egg production using one-way analysis of variance confirmed that there were significant differences between the number of eggs laid by the two weevil species ($F_{318, 1} = 4.26$; p < 0.05) and for the log-transformed data ($F_{318, 1} = 6.01$; p < 0.05). A two-sample t-test showed that there was a significant (P<0.05) difference in the mean number of eggs laid by the two weevil species with *Neochetina bruchi* laying more eggs (292) than *N. eichhorniae* (236) cumulatively.

The effect of time was analyzed for each species using regression of the number of eggs on the time in weeks: The results for *N. bruchi* are shown below:

$$F = 33.4 - 1.79X$$

Where F is fecundity and

X is Weeks

With the following regression diagnostics: Constant (t = 7.89; p < 0.05) and Weeks (t = 4.09; p<0.01). This regression analysis showed that the fecundity of *N. bruchi* significantly decreases with time in weeks. The coefficient of determination was 54.4%, showing that 54.4% of the decrease in fecundity could be explained by the time factor (weeks). The regression line was itself significant with an $F_{14, 1}$ of 19.69 and p-value of 0.001.

For *N. eicchorniae*, the relationship between fecundity (F) and time in weeks could be best described by the following linear equation:

$$F = 28.4 - 1.59X$$

Where F is fecundity and

X is Weeks

The regression diagnostics were: Constant (t = 6.26; p<0.0005) and Weeks (t = 3.40.09; p=0.004), while the coefficient of determination was 45.3%. Only 45.3% of reduction in fecundity for *N. eichhorniae* could be explained by variation in time (weeks). The regression line was also significant with an $F_{14, 1}$ of 11.58 and p-value of 0.004.

The mean number of eggs laid by *N. bruchi* was generally above 20 for the first 10 weeks of the study. The number of eggs laid by this species decreased steadily in the 11th week and by the 16th week, an adult female laid only 2 eggs on average. For *N. eichhorniae*, egg laying pattern was more irregular with a mean laying rate of 11-31 eggs per week for the first 10 weeks. By the 11th week, the mean egg laying per female had reduced to only 8 and by the 16th week, *N. eichhorniae* females were not laying any eggs (Table 4.1).

Table 4. 1: Mean \pm SE and cumulative number of eggs laid by N. bruchi and N. eichhorniae over a 16-week experimental period at KARI, Kibos 2001

| | Mean number of eggs per | | Cumulative egg laying | |
|-------|-------------------------|----------------|-----------------------|----------------|
| Weeks | female per week | | | |
| | N. bruchi | N. eichhorniae | N. bruchi | N. eichhorniae |
| 1 | 19 ± 4 | 11 ± 2 | 19 | 11 |
| 2 | 25 ± 3 | 27 ± 3 | 44 | 38 |
| 3 | 24 ± 3 | 22 ± 2 | 68 | 60 |
| 4 | 22 ± 3 | 23 ± 3 | 90 | 83 |
| 5 | 31 ± 3 | 14 ± 2 | 121 | 97 |
| 6 | 31 ± 6 | 22 ± 4 | 152 | 119 |
| 7 | 35 ± 6 | 31 ± 4 | 187 | 150 |
| 8 | 21 ± 3 | 20 ± 4 | 208 | 170 |
| 9 | 32 ± 5 | 31 ± 3 | 240 | 201 |
| 10 | 21 ± 3 | 21 ± 3 | 261 | 222 |
| 11 | 12 ± 2 | 8 ± 2 | 273 | 230 |
| 12 | 6 ± 2 | 3 ± 1 | 279 | 233 |
| 13 | 3 ± 1 | 1 ± 1 | 282 | 234 |
| 14 | 5 ± 2 | 1 ± 1 | 287 | 235 |
| 15 | 3 ± 1 | 1 ± 1 | 290 | 236 |
| 16 | 2 ± 1 | 0 | 292 | 236 |

The cumulative number of eggs laid by *N. bruchi* reached 292 by the 16th week while the number laid by *N. eichhorniae* reached 236 during the same period of time (Fig 4.1). The overall daily oviposition rate for *N. bruchi* (2.6) was higher than that of *N. eichhorniae* (2.1).

The egg laying capacity shows an initial increasing trend up to the 7^{th} week for both species and then a general decline for both species up to the 16^{th} week (Fig 4.2). The regression equations indicate that *N. bruchi* egg laying would completely cease by the 19^{th} week while that of *N. eichhorniae* would completely cease by the 18^{th} week.

The differences between replicate observations (individual females) was insignificant (P>0.05) for both *N. bruchi* and *N. eichhorniae*.

4.4.2 Life cycle and development time

The generation time of these weevils took 73 and 94 days for *N. bruchi* and *N. eichhorniae* respectively with the duration (days) of the distinct developmental stages as shown in Table 4.2. The durations taken by each developmental stage in both species were compared using a two sample t- test. The mean egg to larva duration was the shortest in both species taking 11 ± 0.2 days in *N. bruchi* and 13 ± 0.44 days in *N. eichhorniae*. These were not significantly different (P<0.05). The larval development stage took the longest time spanning 55 ± 0.79 days in *N. eichhorniae* and 31 ± 0.31 days in *N. bruchi*. This was significantly different (P<0.05). The physically inactive pupal stage took significantly (P<0.05) longer duration of 31 ± 0.49 days in *N. bruchi* compared to 25 ± 0.86 days in *N. eichhorniae*.

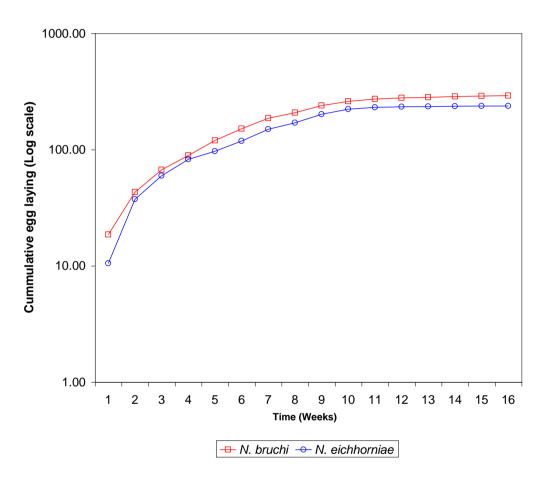


Figure 4. 1: Cumulative egg production (log scale) by *N. bruchi* and *N. eichhorniae* over a 16 weeks experimental period at KARI, Kibos 2001

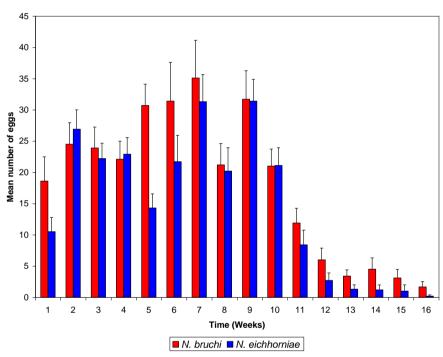


Figure 4. 2: Mean fecundity of *N. bruchi* and *N. eichhorniae* over a 16 weeks experimental period at KARI, Kibos 2001

Table 4. 2: Developmental duration for *Neochetina* weevils' life stages

| Life stage | N. bruchi | N. eichhorniae | Significance |
|-----------------|---------------|----------------|--------------|
| | $Mean \pm SE$ | $Mean \pm SE$ | (P>0.05) |
| Egg | 11±0.2 | 13±0.44 | NS |
| Larva | 31±0.31 | 55±0.79 | * |
| Pupa | 31±0.49 | 25±0.86 | * |
| Generation Time | 74 | 93 | |

4.4.3 Effect of Temperature

During the period of these studies, the mean weekly ambient temperature varied from a

minimum of 21.44°C (week 11) to a maximum of 23.78°C (week 8) thus giving a range

of 2.37°C. A graphical trend of egg laying versus temperature for the *Neochetina* weevils

is shown in Figure 4.3.

From these results, it was possible to determine the relationship between ambient

temperature and fecundity in both N. bruchi and N. eichhorniae. For N. bruchi, the

relationship was weak, with coefficient of determination of only 28.69% (Fig. 4.4).

Temperature was found to be directly related to fecundity according to the equation:

F = 9.2X - 196.33

Where F is Fecundity and

X is Temperature.

For *N. eichhorniae*, the relationship was slightly better than *N. bruchi*, with coefficient of

determination of only 37.78% (Figure 4.5). Temperature was also found to be directly

related to fecundity according to the equation:

F = 10.8X - 229.95

Where F is Fecundity and

X is Temperature

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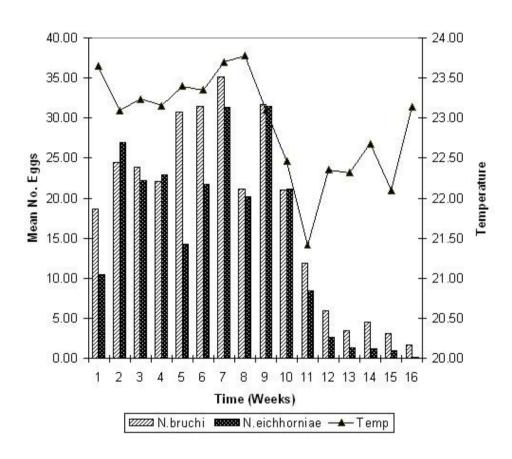


Figure 4. 3: Mean fecundity of *Neochetina* weevils vs Temperature (°C) over 16 weeks at KARI, Kibos, 2001.

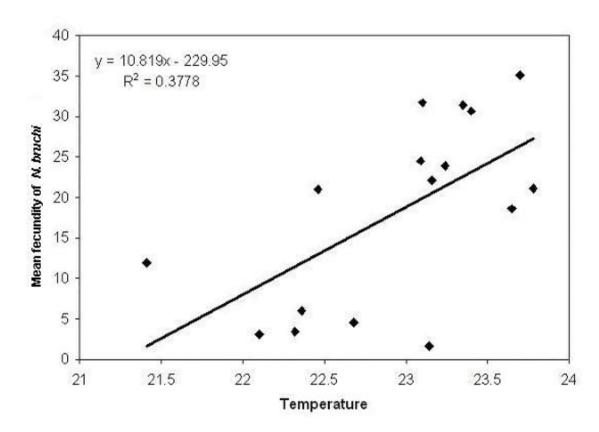


Figure 4. 4: Mean fecundity of *N. bruchi* weevils vs Temperature (°C) over 16 weeks at KARI, Kibos, 2001.

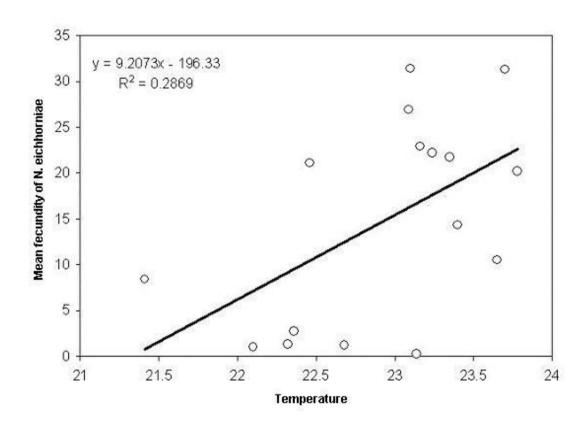


Figure 4. 5: Mean fecundity of *N. eichhorniae* weevils vs Temperature (°C) over 16 weeks at KARI, Kibos, 2001.

4.5 Discussion

The fecundity of the two species *N. bruchi* and *N. eichhorniae* was shown to be significantly (p<0.05) different with *N. bruchi* laying more eggs (292) than *N. eichhorniae* (236). The former also had a higher oviposition rate at 2.6 eggs per day compared to 2.1 eggs for *N. eichhorniae*. Based on these fecundity factors, it seems logical to infer that *N. bruchi* is a more prolific producer of eggs than *N. eichhorniae* in the region under study. Ochiel and Njoka (1999) however noted that despite the higher egg production by *N. bruchi*, it was always out competed in numbers by *N. eichhorniae*, which is not fastidious in its diet. Under natural conditions, *N. bruchi* is a more fastidious feeder and is soon out competed by *N. eichhorniae*, which has no preference for succulent short bulbous petioles. Results of this study compare well with the work of Ogwang and Molo (1997) in Uganda.

The approximate duration (days) of the life stages of these weevils in Argentina and Uganda compared to the present studies in Kenya are presented in Tables 4.3 and Table 4.4. For the species *N. bruchi*, the egg stage took a shorter duration (7.6 days) in Argentina (DeLoach and Cordo, 1976) compared to 11 days in Uganda (Ogwang and Molo, 1997) and 11 days in Kenya (This study). The larval duration, the most important in biological control of the weed, took between 31 days in Kenya, compared to 32 days in Argentina and the longest duration was recorded for Uganda at 35 days. The physically inactive stage of pupa took 30 days in Argentina, 31 days in Kenya and 33 days in Uganda. The total generation time took longest in Kenya at 73 days while it took 69.6 days and 72 days in Argentina and Uganda respectively (Table 4.3). It would appear that the life cycle *N. bruchi* compare favourably and are

similar in the 3 countries, making it easier to import them from Argentina and Uganda for biological control use against water hyacinth in the Kenyan part of Lake Victoria.

For the species *N. eichhorniae* (Table 4.4), the egg stage took 7-14 days in Argentina while it took 10 days in Uganda and 14 days in Kenya. De Loach and Cordo (1976) describe a long duration of between 75 –90 days for the larval stage of this species in Argentina while it took 58 and 55 days only in Uganda and Kenya respectively. The otherwise inactive pupa stage took 14-20 days in Argentina and longer durations of 28 and 25 days in Uganda and Kenya respectively. The entire generation time for *N. eichhorniae* took longest in Argentina (96-120 days), which is more or less similar to that in Uganda and Kenya where it took 96 and 94 days respectively. Thus although Argentinean conditions may be markedly different from that in the East African countries the similarity observed in the generation time may make it easier for *N. eichhorniae* to adapt well and be used for biological control of water hyacinth in any of these countries.

Table 4. 3: Approximate duration (days) of life stages of *Neochetina bruchi* in Argentina, Uganda and Kenya

| Stage | Argentina ¹ | Uganda ² | Kenya ³ |
|-----------------|------------------------|---------------------|--------------------|
| Egg | 7.6 | 7 | 11 |
| Larva | 32 | 35 | 31 |
| Pupa | 30 | 33 | 31 |
| Generation Time | 69.6 | 72 | 73 |

Source: ¹DeLoach and Cordo (1976); ²Ogwang and Molo (1997). ³Present studies

Table 4. 4: Approximate duration (days) of life stages of *Neochetina* eichhorniae in Argentina, Uganda and Kenya

| Stage | Argentina | Uganda | Kenya ³ |
|-----------------|-----------|--------|--------------------|
| Egg | 7-14 | 10 | 14 |
| Larva | 75-90 | 58 | 55 |
| Pupa | 14-20 | 28 | 25 |
| Generation Time | 120 | 96 | 94 |

Source: ¹DeLoach and Cordo (1976); ²Ogwang and Molo (1997). ³Present studies

While there was no significant (p>0.05) difference in the egg to larvae durations for the two weevil species, there was however a significant difference (p<0.05) in the durations of the larva and pupal stages of these two species in the region under study (Table 4.4). It is apparent that the larva causes the most damage to the water hyacinth plant through tunneling the petioles leading to death of the plant. A longer larva duration would therefore predispose the plant to increased exposure to the damaging stage of the insect and may therefore lead to faster death. The species *N. eichhorniae* would thus score higher in the choice of a natural enemy since its larval stage takes 55 days which is significantly (P<0.05) higher than that recorded for *N. bruchi* (31 days).

It is evident from these results that egg laying in both weevil species is irregular during oviposition period. The results also show that there were significant differences in the mean number of eggs laid by the two weevil species as shown in Table 4.1. The mean eggs laid by *N. bruchi* (18.2) was significantly higher than that of *N. eichhorniae* (14.8). Thus the former species could be a better candidate for water hyacinth control due to the larger number of eggs laid. However, using both species for biological control would probably produce synergistic effect against water hyacinth in the Lake Victoria basin.

The results showed that egg laying reduces drastically after the 10th week under semiartificial conditions (Fig 4.2). The implication of such reduction has not also been evaluated in the field. In such a situation, it might be necessary to enhance egg laying by re-inoculation using fresh weevils both in the field and under experimental conditions. Elsewhere, the adults of *N. bruchi* lived for 89 and 134 days in Argentina and India laying a total of 293 and 682 eggs respectively while *N. eichhorniae* lived for 142 days in India and laid 891 eggs. The generation time for *N. bruchi* was observed to be 96 and 72 days in Argentina and Uganda while *N. eichhorniae* took 120 and 96 days respectively (DeLoach and Cordo, 1976a; Jayanth, 1987; Harley, 1990; Ogwang and Molo, 1997).

In the present studies, the adult weevils lived on average for 112 days (16 weeks) laying a total of 292 and 236 eggs for *Neochetina bruchi* and *N. eichhorniae* respectively. The trend of egg laying did not seem to follow the narrow temperature range of 21.5°C to 24.0°C (Fig 4.3). This environmental factor may therefore not be critical in the tropical region.

From the results, it can be estimated that the maximum number of eggs that can be laid by *N. bruchi* in the first week is 31 while that of *N. eichhorniae* is 27 per female. With this kind of information, it is possible to estimate how many weevils are required to effectively control a unit area of water hyacinth infestation within a given time period. For effective implementation, the infestation rate of water hyacinth by the weevils is also required. Julien *et al.* (1999) estimated that a critical threshold of 5 weevils per plant is required to maintain adequate control of the weed. It is important to select newly emerged adults for release, as older ones may have laid all their eggs thereby rendering them ineffective.

The egg-laying in both species completely ceases within 18th or 19th week, after which the adults would not be useful any more for the control of water hyacinth. It is mainly

the larvae that damage the petioles while the adults feed on the leaves leaving scars which, besides reducing the photosynthetic area, predisposes the plant to opportunistic fungi. A monitoring strategy is therefore required on the population dynamics of both species under field conditions. Determining the rates of reproduction and recolonization of the water hyacinth plants can help in developing a control model based on the weevils alone or in combination with other control agents and methods.

CHAPTER FIVE

5.0 Life Tables and Survivorship for *Neochetina* weevils on Water Hyacinth

5.1 Introduction

A "life table" is a listing of the number or densities in a population surviving to specific ages or stages in the life cycle. Life tables were originally constructed by insurance companies as a means of determining the relationship between age and the potential for a client surviving to pay sufficient insurance premiums to keep the company solvent (Elkinton, 1993). These insurance life tables provide some basic information on survival but ignore the process of birth. By expanding life tables to include information on fecundity (birth rate), age group and mortality, it gives an effective means of organizing demographic data (Barbour *et al.*, 1987). Morris and Miller (1954), studying the spruce budworm, *Christoneura fumiferana* in Canada were the first to utilize the life table format for the study of insect populations.

The construction of life tables is an important component in the understanding of the population dynamics of a species. Although some animal ecologists, such as Richards (1940), had expressed their results showing the successive reductions in the population of an insect throughout a single generation, Deevy (1947) was really the first worker to focus attention on this approach while studying the Dall sheep, *Ovis dalli* in Alaska. He defined life tables as "a concise summary of certain vital statistics of a population". They are fundamental requisites to the analysis of population processes and to understanding population dynamics. Analysis of the existing natural

mortality and knowledge of population dynamics are prerequisites to conducting a successful biological control programme (Southwood, 1978; Harley, 1990).

5.1.1 Types of Life Tables

Deevy (1947) and Southwood (1978) recognized two types of life tables. An "age specific", "horizontal" or "cohort life table" tabulates the survival of a cohort of individuals (all born at the same time) as they age. Such life tables are principally used for insect species with discrete generations. The majority of species in this concave type of survivorship, with more than 70% mortality occurring prior to the mid-larval stages, are free living with a physically exposed habitat, although some are in a cryptic habitat (Price, 1975).

"Vertical life tables", also known as "static" or "time specific", are based on the fate of an imaginary cohort found by determining the age structure, at point of time, of a sample of individuals from what is assumed to be a stationary population with considerable overlapping of generations, i.e. a multistage population (Southwood, 1978). Age determination is a prerequisite for time specific life tables. Vertical life tables provide an instantaneous picture of the age structure of a population, appropriate for populations with overlapping generations. Such an age structure reflects the mortality that occurs at each age and, provided the intrinsic rate of natural increase (*r*) is close to zero, the age structure approximates the probability of survival of cohorts (Caughley, 1977). This second type is characterised by a convex survivorship curve with 60% or more mortality occurring after the mid-larval stages. The majority of species in this type appear to be protected by a burrowing habit or

colonial defence behaviour (Price, 1975). Such life tables also typically list the age specific fecundity in addition to survival (Elkinton, 1993; Southwood, 1978).

5.1.2 Survivorship Curves

A "survivorship curve" is a graphical representation of the tabular life table in which the number surviving to each age category in a population is plotted on a log scale (Pearl, 1928) or on absolute numbers (Slobodkin, 1962) against age. In insects, mortality often occurs in distinct stages so that the survivorship curves show a number of distinct steps (Ito, 1961).

Different animal species exhibit different characteristically shaped survivorship curves (Fig 5.1). In a type I curve, the rate of mortality increases at old age. Human beings have a survivorship curve that is close to Type I. A type II curve is linear (provided the number surviving is plotted on a log scale), representing an exponential decline in numbers. In type II, the rate of mortality is constant throughout life i.e. the probability of dying is essentially the same at any age. Some birds exhibit this type of curve. In the Type III survivorship, the greatest mortality rates occur in the youngest age category. Most insects and other invertebrates exhibit a Type III survivorship (Southwood, 1978).

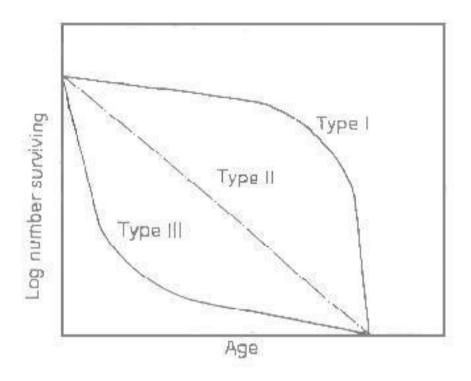


Figure 5. 1:Types of Survivorship Curves.

Number surviving (on a log scale) to successive ages for species in which mortality rates increase (Type I), decrease (Type III), or remain constant (Type II) with age. (Adopted from Elkinton, 1993)

5.2 Objective

- 1. To determine the survivorship of the various life stages of *Neochetina* weevils on water hyacinth under different egg setting methods.
- 2. To compare the survival and mortality of the 2 weevil species using life tables and survival curves.

5.3 Hypotheses

- 1. There is no significant difference in the survival and mortality of *Neochetina* weevils under different egg setting methods.
- 2. The two weevil species, *Neochetina bruchi* and *N. eichhorniae* do not exhibit similar survivorship curves.

5.4 Materials and Methods

5.4.1 Source of eggs

For *N. bruchi* and *N. eichhorniae*, twenty freshly emerged adult mating pairs were each put in thirty 500 ml clear plastic containers (n=600) and provided with 3 water hyacinth leaves plus three 5 cm long petioles cut on both ends for feeding and oviposition respectively. Some water (100 ml) was provided to avoid desiccation. These containers were then secured on a wooden bench in an open-air laboratory at Kibos. The required number of eggs for survival and mortality studies were harvested from the containers with the respective species.

5.4.2 Survival of *Neochetina* weevils under different egg setting methods

5.4.2.1 Incision Egg Setting (IES)

In this method, 9 plastic buckets each with a capacity of 20 litres were filled ¾ full with water and some 3 healthy water hyacinth plants introduced into each. Sets of 100 eggs borne on 2mm petiole discs (each having 10-20 eggs) were harvested from the respective species containers. The discs were inserted at about mid-length of the petioles using sterile scalpels to make the incisions. The experiment was set in a Complete Randomised Design and replicated nine times. Each bucket was then covered with an insect proof muslin cloth mounted on 75 x 75 cm wooden cage (Plate 5.1).



Plate 5. 1 Insect proof cage used in the Experiments at KARI, Kibos.

The plants were observed daily for signs of larval tunnelling. After 30 days, the plants were carefully dissected using a scalpel to recover the emerged third instar larvae without killing them (non destructive sampling). These were counted and recorded for each container. The petiole sections with the recovered larvae were put back into the respective buckets complete with fresh water and new plants for pupation.

After 30 days pupation on the hair roots was examined for both species. The pupal cocoons were counted cumulatively until there was no increase in their numbers. They were then left to develop into adults. The emerging adults were counted and removed from the containers immediately until no new ones were emerging.

5.4.2.2 Free Egg Setting (FES)

In this method, 9 plastic buckets each with a capacity of 20 litres were filled ¾ way with water and some 3 healthy water hyacinth plants introduced into each. Sets of 100 eggs borne on 2mm petiole discs (each having 10-20 eggs) were harvested from the respective species containers. The procedure in this method of egg setting is similar to the IES above except that the discs bearing the egg batches are not incised into the plant but left to float freely in the buckets containing the water hyacinth plants. The hatched larvae in this method do not immediately enter into the petiole as in the incision method where the eggs are set inside the petiole. The experiment was set in a Complete Randomised Design and replicated nine times. Each bucket was then covered with an insect proof muslin cloth mounted on 75 x 75 cm wooden cage (Plate 5.1).

5.5 Results

5.5.1 Survival and Life tables

5.5.1.1 Eggs to Larvae

The experiment used 100 eggs per cohort in 9 replicates thus 900 eggs were examined for each egg setting method. Table 5.1 presents the data obtained for *Neochetina bruchi*. It was found that out of 100 eggs the mean number of larvae obtained were 33.8 ± 6.0 for the Incision Egg Setting (IES) method as compared to only 18.7 ± 2.6 in the Free Egg Setting (FES) method. These results were significantly different (p<0.05) with the IES method yielding higher number of larvae and providing better opportunities for use in biological control programmes.

It was also found that the IES method had a significantly (p<0.05) lower egg to larvae loss (mortality) at 66.2% than the FES method, which scored a loss of 81.3%. Thus the former is a better technique for mass rearing of these weevils.

In the case of *N. eichhorniae* (Table 5.2), it was found that out of 100 eggs the mean number of larvae obtained were 25.4 ± 4.6 for the Incision Egg Setting (IES) method as compared to only 19.7 ± 4.1 in the Free Egg Setting method. The differences in the mean number of larvae yielded in the two methods of egg setting were significantly (p>0.05) different with the IES method yielding a higher number of larvae. The mortality loss recorded for this stage in the IES was significantly (p>0.05) lower (74.6%) than the loss occasioned in FES method (80.3%).

5.5.1.2 Larvae to Pupae

The mean number of *N. bruchi* pupae recorded for the Incision Egg Setting method (14.2 \pm 2.0) was not significantly (p>0.05) higher than the number yielded by the Free Egg Setting method (12.4 \pm 1.1) as shown in Table 5.1.

There was a significant difference (p>0.05) in percent mortality loss in the two methods of egg setting with the IES posting a higher (57.9%) loss than FES (33.6%) for this developmental stage.

For *N. eichhorniae*, the mean number of pupae yielded in the IES method (10.1 ± 1.8) was not significantly (p>0.05) higher than the number recorded from the FES method (7.5 ± 1.5). The percent mortality loss recorded for this stage was not significantly different (p>0.05) at 60.2% and 61.9% for IES and FES respectively as shown in Table 5.2.

5.5.1.3 Pupae to Adults

The mean number (11.7 ± 2.5) of *Neochetina bruchi* adults yielded in the IES method was not significantly (p>0.05) higher than the number (10.8 ± 1.4) yielded in the FES method. For the mortality loss, there was no significant difference (p>0.05) in the two methods of egg setting at 17.6% for IES and 12.9% for FES (Table 5.1). In the case of *N. eichhorniae*, there was also no significant difference (p>0.05) between the IES (6.4 ± 1.4) and FES (5.5 ± 1.2) pupa to adult yields. The percent mortality loss for this stage was significantly different (p>0.05) at 36.6% and 26.6% for IES and FES respectively (Table 5.2).

Table 5. 1: Mean survival and mortality of *N. bruchi* under Incision (IES) and Free Egg Setting (FES) methods at KARI, Kibos, 2001 (9 replicates of 100 egg cohort each).

| Life | Mean surv | vivors ± SE | Log No. Sur | viving | Number los | st per stage | % Loss | s per stage |
|--------|----------------|----------------|-------------|--------|------------|--------------|--------|-------------|
| Stage | IES | FES | IES | FES | IES | FES | IES | FES |
| Eggs | 100 | 100 | 2 | 2 | | | | |
| Larvae | 33.8 ± 6.0 | 18.7 ± 2.6 | 1.5 | 1.27 | 66.2 | 81.3 | 66.2 | 81.3 |
| Pupae | 14.2 ± 2.0 | 12.4 ± 1.1 | 1.15 | 1.09 | 19.6 | 6.3 | 57.9 | 33.6 |
| Adults | 11.7 ± 2.5 | 10.8 ± 1.4 | 1.06 | 1.03 | 2.5 | 1.6 | 17.6 | 12.9 |

Table 5. 2: Mean survival and mortality of *N. eichhorniae* under IES and FES methods at KARI, Kibos, 2001 (9 replicates of 100-egg cohort each).

| Life | Mean surv | vivors ± SE | _ | No. | Number lost per | stage | % Loss | s per stage |
|--------|----------------|----------------|------|------|-----------------|-------|--------|-------------|
| Stage | IES | FES | IES | FES | IES | FES | IES | FES |
| Eggs | 100 | 100 | 2 | 2 | | | | |
| Larvae | 25.4 ± 4.6 | 19.7 ± 4.1 | 1.40 | 1.29 | 74.6 | 80.3 | 74.6 | 80.3 |
| Pupae | 10.1 ± 1.8 | 7.5 ± 1.5 | 1.00 | 0.87 | 15.3 | 12.2 | 60.2 | 61.9 |
| Adults | 6.4 ± 1.4 | 5.5 ± 1.2 | 0.80 | 0.74 | 3.7 | 2.0 | 36.6 | 26.6 |

5.5.2 Survival Curves

5.5.2.1 Neochetina bruchi

The survival curve for *N. bruchi* (Fig. 5.2) shows that the highest mortality is realized in the younger stages of the weevil development from egg to larvae where the numbers were reduced from 100 to 18.7 (free egg setting) and 33.8 (incision egg setting) resulting in 81.3% and 66.2% mortality respectively. From larvae to pupa, there was a mortality of 12.4 (free egg setting, 33.6%) and 14.2 (incision egg setting method, 57.9%). For the pupa to adult stage, the mortalities were 12.9% (free egg setting) and 17.6% (incision egg setting).

5.5.2.2 Neochetina eichhorniae

The survival curve for *N. eichhorniae* (Fig.5.3) shows that the highest mortality is realized in the younger stages of the weevil development from egg to larvae where the numbers were reduced from 100 to 19.7 (free egg setting) and 25.4 (incision egg setting). These figures resulted in 80.3% mortality and 74.6% mortality respectively.

From larvae to pupa, there was a mortality of 12.2 (free egg setting, 61.9% mortality) and 15.3 (incision setting method, 60.2%). During the last developmental stage from pupa to Adult, the mortalities were reduced to 3.7 (36.6%) in the IES while in the FES the loss was 2.0 (26.6%).

5.5.2.3 Free Egg Setting Method for Neochetina weevils

Using the Free Egg Setting method, it could be shown that the survival rate of *N. bruchi* and *N. eichhorniae* are similar from the egg stage to larval stage (Fig. 5.4). However, from the larval stage to pupa, *N. bruchi* showed higher survival than *N. eichhorniae*, indicating that *N. bruchi* is better candidate for biological control of water hyacinth since the larval is the most destructive stage of these insects.

Further, superior survival of *N. bruchi* from pupa to adult as compared to *N. eichhorniae* offers it more opportunity and capacity for self regeneration, thereby ensuring better continuity over the generations.

5.5.2.4 Incision Egg Setting Method for Neochetina weevils

Using the Incision Egg Setting method, it could be shown that the survival rate of *N. bruchi* was higher than that of *N. eichhorniae* for all developmental stages (Fig. 5.5). In this case again, *N. bruchi* is a better candidate for biological control of water hyacinth as compared to *N. eichhorniae*.

5.5.2.5 Two-way comparison of egg setting methods with weevil species

A comparison of the mean survival in numbers by Two-Way ANOVA showed that there was significant difference in the survival based on the method of incubation from egg to larval stage (p=0.001). The Incision Egg Setting method was superior

with an average of 53.4 as compared to Free Egg Setting method (29.9). *Neochetina bruchi* had a significantly higher survival from larva to pupa stage (p=0.003). The detailed analysis of the incubation methods and species survival is shown in Table 5.3.

5.5.3 Survival rates

The survival rate for these weevils was also calculated using the Ricker (1975) formula. The rate took into consideration the time taken to pass from one developmental stage to the next. For N. bruchi, the survival rates were significantly different (p>0.05) between the two methods of egg setting for all the developmental stages (Fig 5.6). Similarly, there was significant difference (p>0.05) between all the developmental stages of N. eichhorniae for both methods of egg setting (Fig.5.7).

When the annual survival rate was considered, after taking into account the time taken to pass through each distinct developmental stage, the annual survival rates were found to be slightly lower for the early developmental stages of egg to larvae for N. bruchi (free egg setting = 0.863 and incision setting = 0.908).

The annual survival rate from larvae to pupa for *N. bruchi* was 0.952 (FES) and 0.927 (IES). From pupa to adult stage, the survival rate for *N. bruchi* was 0.993 (FES) and 0.989 (IES) as shown in Fig. 5.6.

Similarly, the annual survival rates were also found to be slightly lower for the early developmental stages for N. eichhorniae (FES= 0.866 and IES = 0.884). Survival rate from larvae to pupa for N. eichhorniae was 0.887 (FES) and 0.904 (IES). From pupa

to adult stage, the survival rate for *N. eichhorniae* was 0.975 (FES) and 0.976 (IES) as shown in Fig. 5.7.

Thus in all cases it may be concluded that the survival rates generally increased with higher developmental stages i.e. mortality was higher in the lower developmental stages thus: eggs > larvae > pupae > adults (Table 5.4).

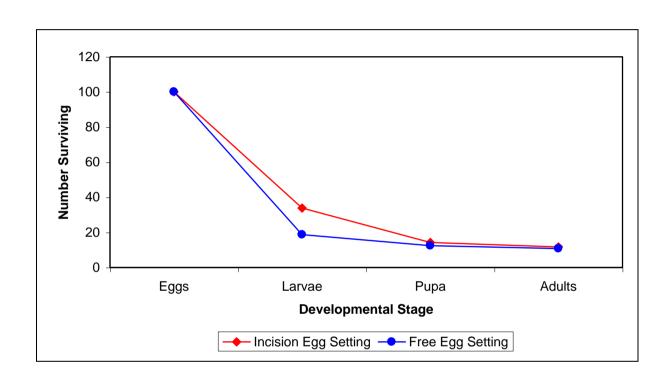


Figure 5. 2: Survival curve of $N.\ bruchi$ under two methods of Egg Setting at KARI, Kibos, 2001

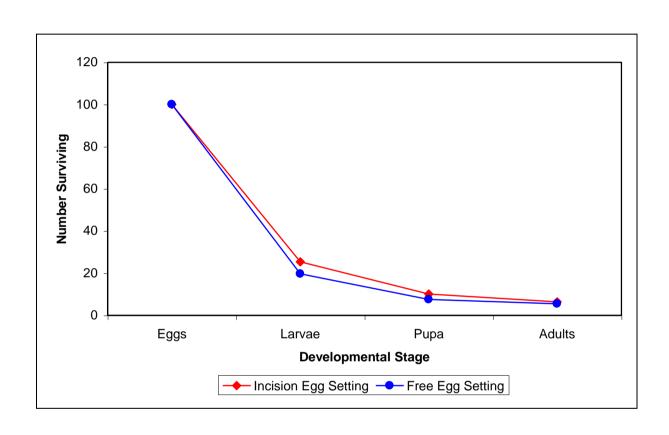


Figure 5. 3: Survival curve of *N. eichhorniae* under two methods of Egg Setting at KARI, Kibos, 2001

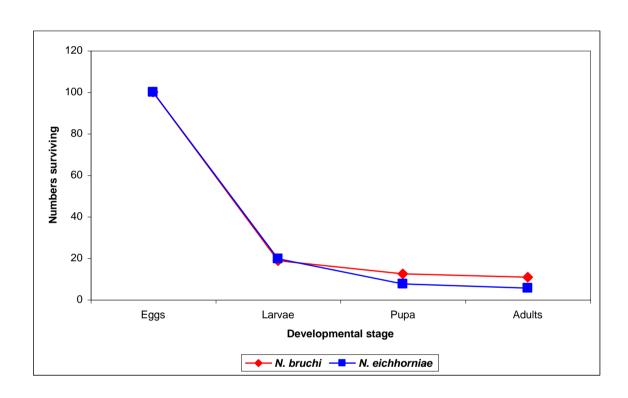


Figure 5. 4: Survival curve of *Neochetina* weevils under Free Egg Setting Method at KARI, Kibos 2001

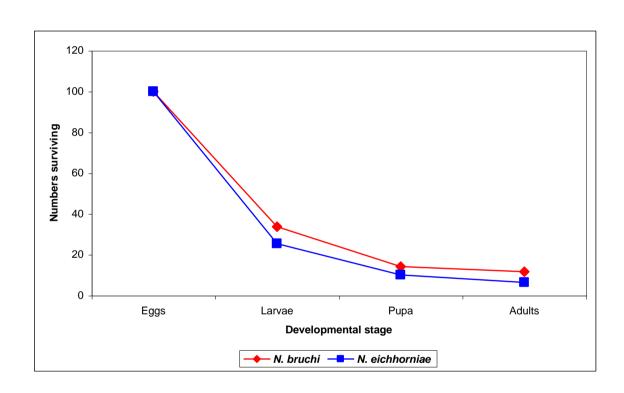


Figure 5. 5: Survival curve of *Neochetina* weevils under Incision Egg Setting Method at KARI, Kibos 2001

Table 5. 3: Two-way ANOVA results for survival of the weevils classified by species / method used for incubation and mean values for species / method

| Life stage | Source of variation | F | P | | ues | | |
|-------------|---------------------|-------|-------|-----------|----------------|------|------|
| | | | | N. bruchi | N. eichhorniae | FES | IES |
| Egg-Larvae | Species | 2.35 | 0.135 | 46.7 | 36.7 | 29.9 | 53.4 |
| | Method | 13.05 | 0.001 | | | | |
| | Interaction | 8.39 | 0.007 | | | | |
| Larvae-Pupa | Species | 10.01 | 0.003 | 25.6 | 17.1 | 21.4 | 21.2 |
| | Method | 0.01 | 0.918 | | | | |
| | Interaction | 6.47 | 0.016 | | | | |
| Pupa-Adult | Species | 0.05 | 0.823 | 9.22 | 8.94 | 9.5 | 8.67 |
| | Method | 0.46 | 0.504 | | | | |
| | Interaction | 9.67 | 0.004 | | | | |

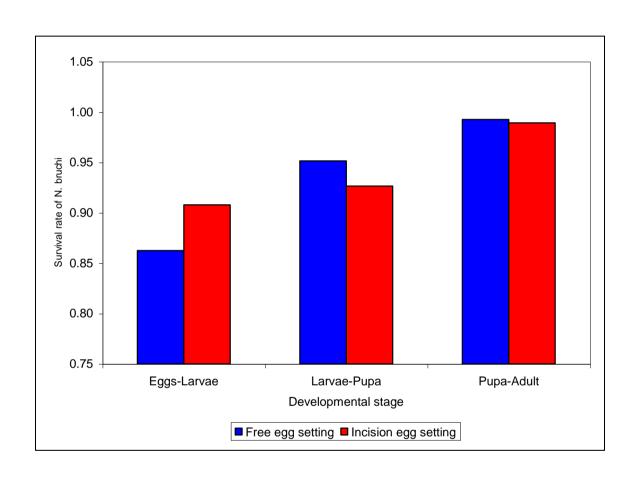


Figure 5. 6: Survival rate of N. bruchi under 2 methods of egg setting

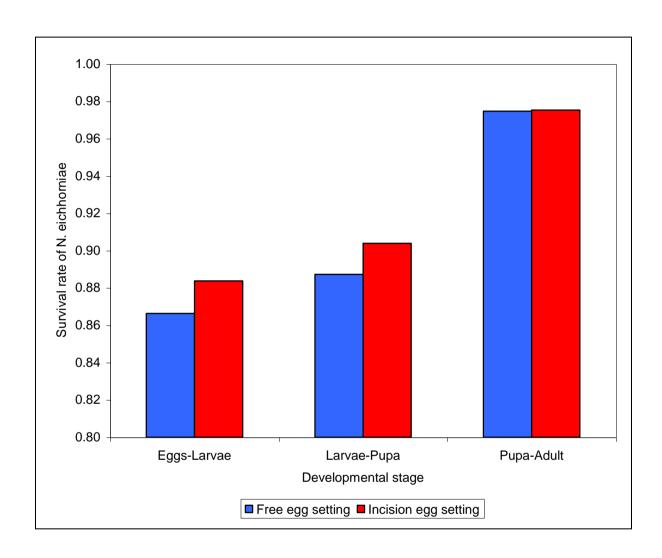


Figure 5. 7: Survival rate of N. eichhorniae under 2 methods of egg setting

Table 5. 4: Survival Rates for *Neochetina* weevils under 2 methods of Egg Setting at KARI Kibos, 2001

| Life Stage | N. bruch | ni | N. eich | horniae |
|----------------|----------|-------|---------|---------|
| Life Stage | IES | FES | IES | FES |
| Egg to Larvae | 0.908 | 0.863 | 0.884 | 0.866 |
| Larvae to Pupa | 0.927 | 0.952 | 0.904 | 0.887 |
| Pupa to Adult | 0.989 | 0.993 | 0.976 | 0.975 |
| | | | | |

ANOVA

| | Sp | ecies | Method | | |
|----------------|--------------|---------|--------------|---------|--|
| Egg to Larvae | F1,33 = 0.72 | p=0.402 | F1,33 = 4.14 | p=0.049 | |
| Larvae to Pupa | F1,33 = 1.12 | p=0.298 | F1,33 = 5.25 | p=0.029 | |
| Pupa to Adult | F1,33 = 1.96 | p=0.171 | F1,33 = 1.29 | p=0.265 | |

5.6 Discussion

Results obtained for the survival studies indicate that there was high mortality for both *N. bruchi* and *N. eichhorniae* at the early life stages. However, mortality rates declined exponentially with time for both the species. Method of egg incubation seemed to be important in the early life stages. This observation is expected since many arthropod species experience high mortalities in their early life stages. As can be seen from Figures 5.1 and 5.2 for both species, the early life stage of the two species showed dramatic loss of individuals which was further affected by the method used for incubation as compared to later (larvae to adult) stage survival. This situation indicates the desire for greater technical care to avoid losses (high mortality) in the early life stage when releasing the biological control agents on water hyacinth.

From the results of survival rates (Table 5.3), it was clear that there were no significant differences in the survival rate of both species of the weevils from egg to larval stage. This is the most critical stage for biological control of water hyacinth. It is therefore possible to use either of the species for the control of water hyacinth. However, the synergistic effect of both species under field conditions has not been evaluated. Under field conditions, it is necessary to test each species for control independently and also to test both the species in varying proportions before any single species is recommended. Besides, it might not be advisable to recommend a single species for biological control of water hyacinth in the field. Many biological control programmes are known to employ more than one biological agent and also additional non-biological methods to achieve higher IPM degrees of success. In any

case the two species occur together in their native habitat and are effective in suppressing the weed.

The survival phenomenon observed in both species is consistent with the Type III survivorship curve described for most insects and other invertebrates (Southwood, 1978). On average, about 10% of each species reaches the adult stage but this is over a small time period of only 3 months. In the field, the cumulative effect of reinoculation and multiple egg laying may have to be evaluated. On an annual basis, the cumulative survival rate is most likely to be higher than estimated in this semi-artificial environment. According to basic ecological principles, it may also be necessary to use an exponential population model for mortality and survival studies.

When survival rates are considered, using the Ricker (1975) formula, and the fact that these are calculated on an annual basis and by using the exponential population model, it becomes clear that the survival rate of these two weevil species could be higher in the field than under experimental conditions. These values seem to be all above 0.9 except in the incision egg setting for *N. eichhorniae*. The annual survival rate of both the weevil species may therefore not be too low even if mortality in the initial stages of the life cycle is high.

The survival characteristics of both the weevils attest to the fact that these two species have been used for the biological control of water hyacinth in many parts of the world. Whereas semi-artificial conditions may not clearly show such characteristics, field observations have not been made in the Lake Victoria basin and these were indeed pioneer studies and provide significant output for the current use of these

species and future investigations to improve the efficiency of biological control against water hyacinth.

It was also found that the IES technique had a significantly lower egg to larvae loss (mortality) at 66.2 % than the FES technique with 81.3 % loss for *N. bruchi* (p=0.001). On mortality, the IES method posted a lower egg to larvae loss (74.6%) than the FES method at (80.3%). *N. eichhorniae* therefore, showed a similar trend, thereby indicating the superiority of IES over FES in the development of the two weevils from egg to larvae. IES seems to be a better technique for mass rearing of *Neochetina* weevils to obtain a reasonable number of the critical life stage (larvae) for control of water hyacinth, as at now and in the absence of improved techniques.

From larvae to pupa, both methods were not significantly different (p=0.918) but *N. bruchi* posted higher mean values of pupa as compared to *N. eichhorniae*. Since pupa stage is not the critical stage for control of water hyacinth, the high survival might not make *N. bruchi* a better candidate for biological control of water hyacinth.

The survival rates also showed that there were no significant differences between the species (p=0.72 to 1.96) and therefore confirms that both species are good candidates as biological control agents for water hyacinth in the Lake Victoria Basin.

CHAPTER SIX

6.0 Impact of *Neochetina* weevils on water hyacinth in Lake Victoria basin

6.1 Introduction

Water hyacinth is a fast growing plant. In Lake Victoria basin, it has spread to many parts of the lake and in numerous surrounding Small Water Bodies. Control methods other than the use of natural enemies are environmentally unsafe, difficult and unsustainable. Trials using *Neochetina* weevils offer good opportunities for controlling the weed. However, it is not well known how the release of these weevils would cause damage and help reduce invasion by the weed in the basin. Despite the release of the weevils into the lake, their impact, establishment and control of the weed were until the present studies not understood.

Both species of *Neochetina* weevils feed on water hyacinth plants causing similar damage. Adults feed on the leaves causing scars (Plate 6.1). The numerous scars debilitate the plant by removing extensive proportions of epidermal tissues thus increasing water loss and reducing the photosynthetic area. The scars also expose the plant to attack by opportunistic pathogens. Extensive feeding around the upper petiole may girdle the petiole and kill the lamina above (Goyer *et al.*, 1984). The larvae first make a tunnel into the lower petiole and crown, and then damage tissues and buds, initially preventing flowering (Plate 6.2). As damage on the plant increases, growth rate is reduced and the production of new leaves and stolons is reduced (De Loach *et al.*, 1983). Plant size parameters (petiole length, laminar area, fresh weight and stolon length) decline with increasing damage by the weevils. The damage by adults and

larvae causes the lower petioles to rot, water logging of the crown and gradual sinking of the plant. Thus the crown sinks several centimetres below the water surface and the plants subsequently die and sink. The duration from the release of weevils to plant death takes several months depending on a combination of factors, such as temperature, nutrient status of the weed, number of weevils released and their distribution through the infestation (Julien, 2001).

Water hyacinth is an exotic plant native in the Amazonian Brazil. The weed is believed to have gained entry into Lake Victoria through River Kagera. Its spread to the numerous small water bodies in the basin has been through dispersal by man, animals and wind through seeds and live plants. Since the weed reproduces very prolifically in nutrient rich fresh water, it colonized the satellite ponds in the Kenyan basin of Lake Victoria very quickly. There were no deliberate introductions of the weevils in these ponds. However, preliminary survey in the selected study ponds showed the weevils were present albeit in small numbers probably having migrated with the weed from the open lake via the various dispersal mechanisms.

These studies quantified the weevils' impact on the water hyacinth in the selected satellite ponds in the Lake Victoria basin.



Plate 6. 1: Feeding scars caused by adult *Neochetina* weevils

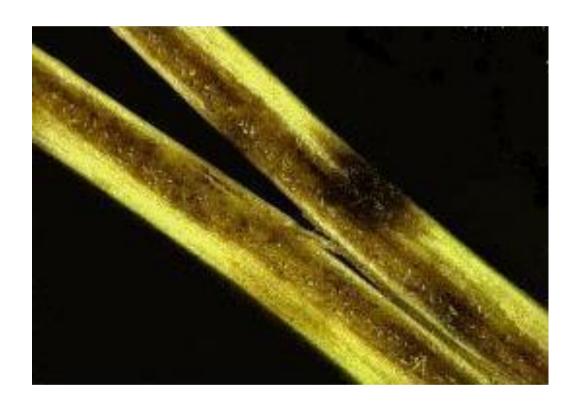


Plate 6. 2: Advanced petiole damage caused by Neochetina larvae

6.2 Objective

- To determine the nature and extent of damage on water hyacinth plants by the *Neochetina* weevils.
- 2. To evaluate the impact of *Neochetina* weevils on water hyacinth in different water bodies in the Lake Victoria Basin.

6.3 Hypotheses

- 1. Different plant parts are not attacked and damaged equally.
- 2. *Neochetina* weevils are not effective biological control agents of water hyacinth.

6.4 Materials and Methods

These studies were conducted at 3 pond sites spread over 3 districts, namely Budalangi (Busia district), Okana (Kisumu district) and Otho (Nyando district). Data was collected over 12 months at monthly intervals at Okana and Otho while at Budalangi it was taken for 6 months as the pond dried up earlier. At each site 3 line transects were randomly selected for sampling. The transects were sited parallel to the shore of the pond. A total of 18 plants were sampled with each transect having 6 plants picked at 1 metre interval. From each plant sampled, data was recorded on the number of ramettes, laminar area, petioles number and length (Julien *et al.*, 1999). The plants were further carefully examined and any damage attributable to the weevils recorded. Such damage included feeding scars on different plant parts (leaves, shoots, stems) and dead or dying shoots.

The sampled plants from Otho and Okana sites were labeled and taken to the laboratory at NFRC Kibos. The plants were then dissected using a scalpel to record the number of eggs, larvae, pupae, adults and damaged petioles. However, for plant samples from Budalangi data on weevil adults were only recorded in the field due to the distance from the laboratory at NFRC Kibos.

6.5 Results

6.5.1 Plant characteristics

At Budalangi study site, there were significant differences between sampling dates for the number of rametes, petiole length, laminar area and the total number of petioles (Table 6.1). The highest mean number of rametes was observed in July (5.0 ± 0.33) and this value was higher than all other values in all the months during the study period. The lowest mean petiole length was also observed in July (10.33 ± 0.42) . The smallest lamina area was in July and August while the lowest number of petioles was observed in June and August.

The number of damaged petioles was highest in the months of July 2001 (4.83 \pm 0.77), August 2001 (4.22 \pm 0.40) and September 2001 (4.94 \pm 0.46). The least number of damaged petioles was observed in May 2002 (1.66 \pm 0.39) but this value was not significantly different from March, April and June 2002. The percentage of damaged petioles did not however follow any specific pattern (Table 6.2).

Table 6. 1: Mean scores $(\pm SE)$ for plant parameters at Budalangi, 2001.

| Month | Rametes | Petiole Length | Laminar Area | Total Petioles |
|--------|---------------------|---------------------------|--------------------------|--------------------------------|
| Month | $(No.) \pm SE$ | $(cm) \pm SE$ | $(cm^2) \pm SE$ | $(No.) \pm SE$ |
| March | 2.72 ± 0.30^{d} | 21.3 ± 1.33 ^a | 56.6 ± 4.41 ^a | 16.72 ± 1.59 ^{cd} |
| April | $4.55\pm0.42^{~ab}$ | 19.1 ± 1.51 ^{ab} | 48.9 ± 5.49 ab | 24.3 ± 2.1 ab |
| May | $4.0\pm0.43b^{bc}$ | 20.1 ± 1.27 ab | 45.4 ± 3.68 b | 21.8 ± 2.35 a |
| June | 3.16 ± 0.18 cd | 15.2 ± 1.19 ° | 48.8 ± 4.64^{ab} | 14.5 ± 1.18 ^{cd} |
| July | 5.0 ± 0.33 a | 10.33 ± 0.42 | 30.9 ± 2.17 ° | 18.6 ± 1.64 bc |
| August | 2.72 ± 0.3^{d} | 17.38 ± 1.14 bc | 39.67 ± 3.1 bc | 12.6 ± 1.16 ^d |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI.

Examination on the damage caused by the weevils showed that the highest number of feeding scars was observed in March (39.2 \pm 6.17) while the highest number of damaged petioles was observed in April, May and July. The highest percentage of damaged petioles however spread throughout the study period from March to July (Table 6.2). Only August had significantly lower percentage of damaged petioles (18.7 \pm 1.46) as compared to all other months.

The number of *N. eichhorniae* showed that they were relatively constant throughout the study period and there were no significant differences between the months (Table 6.3). The numbers of *N. bruchi* showed minimal values in August and highest values in April, May and June. It was noted that *N. eichhorniae* out competed *N. bruchi* in percent occurrence at Budalangi recording 66.4% and 33.6% respectively.

Table 6. 2: Nature and extent of damage by *Neochetina* weevils on water hyacinth given by mean feeding scars and damaged petioles at Budalangi, 2001.

| Month | Feeding Scars(No.) ± | Damaged Petioles | | |
|--------|----------------------|------------------------|--------------------------|--|
| Wionth | SE | Number ± SE | % Damaged ± SE | |
| March | 39.2 ± 6.17 | $5.1 \pm 0.69^{\ b}$ | 31.6 ± 3.41 ^a | |
| April | 22.9 ± 3.98^{a} | $7.55\pm0.69~^{\rm a}$ | $31.8\pm2.27^{\rm \ a}$ | |
| May | 16.6 ± 1.95 ab | $7.5\pm0.93~^{\rm a}$ | 34.9 ± 3.09^{a} | |
| June | 17.4 ± 3.0 ab | 4.66 ± 0.69 b | $33.6\pm4.88^{\rm \ a}$ | |
| July | 8.94 ± 0.78 b | $6.16\pm0.88~^{ab}$ | 31.8 ± 2.91^{a} | |
| August | 14.5 ± 2.52 ab | 2.33 ± 0.22 | 18.7 ± 1.46 | |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI.

Table 6. 3: Adult Neochetina weevils occurrence at Budalangi, 2001.

| Month | Numbe | Total | |
|--------------|-----------------|-----------------|-----|
| | N. bruchi | N. eichhorniae | |
| March | 3 b | 16 a | 19 |
| April | 13 ^a | 14 ^a | 27 |
| May | 7 ab | 13 ^a | 20 |
| June | 10^{ab} | 11 ^a | 21 |
| July | 5 ^b | 16 ^a | 21 |
| August | 1 | 7 ^a | 8 |
| Total | 39 | 77 ^a | 116 |
| % Occurrence | 33.6 | 66.4 | |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI.

At Okana, the number of adult *N. bruchi* observed did not vary much between months but was highest in July 2001 to September 2001 (25 to 27). The highest number of *N. eichhorniae* was observed in July 2001 (66), August 2001 (61) September 2001 (65) and May 2002 (71). The lowest numbers were 18 and 11 in November 2002 and December 2002 respectively (Table 6.5). *N. eichhorniae* was overall the predominant species at 79.6% compared to *N. bruchi* at 20.4% at Okana.

6.5.2 Plant damage

The number of feeding scars recorded at Okana was highest in the months of April 2001 (108.83 \pm 4.69), March (99.8 \pm 4.23) and in May (94.72 \pm 5.14). The number of damaged petioles was highest in January 2002 (5.61 \pm 0.73), September 2001 (4.94 \pm 0.46) and in August 2001 (4.22 \pm 0.40) with a range of values from 1.66 to 5.61 (Table 6.4).

At Otho study site, the number of feeding scars was highest in September 2001 (93.11 \pm 7.91), March 2002 (88.0 \pm 8.11), April 2002 (98.5 \pm 10.11), May 2002 (85.1 \pm 8.13) and June 2002 (94.94 \pm 7.66). The numbers of damaged petioles were highest in July 2001, September 2001, December 2001, January 2002, March 2002 and April 2002 with a range of values from 2.05 to 2.61 (Table 6.6).

Table 6. 4: Nature and extent of damage by *Neochetina* weevils on water hyacinth given by mean feeding scars and damaged petioles at Okana, 2001.

| Month | Feeding Scars | Damaged Petioles | | | |
|--------------|--------------------------------|------------------------------|-----------------------------|--|--|
| Month | $(No.) \pm SE$ | Number ± SE | % damaged ± SE | | |
| July 2001 | 56.27 ± 4.52 ^e | 4.83 ± 0.77^{ab} | 34.59 ± 5.66 ab | | |
| August | 82.44 ± 6.84 ^{cd} | $4.22\pm0.40~^{abc}$ | 28.39 ± 3.12 bc | | |
| September | 91.0 ± 9.08 bc | $4.94\pm0.46^{\rm \ a}$ | $35\pm3.05~^{ab}$ | | |
| October | 52.61 ± 3.95 e | 3.22 ± 0.34 cd | $28.9 \pm 3.6^{\ bc}$ | | |
| November | 34.33 ± 2.65 f | $2.61\pm0.30^{\ d}$ | 21.7 ± 2.96 ° | | |
| December | $36.61 \pm 3.10^{\text{ f}}$ | $3.38\pm0.42^{\ cd}$ | 27.8 ± 3.72 bc | | |
| January 2002 | 75.5 ± 4.24^{d} | $5.61\pm0.73~^{abc}$ | $45.6 \pm 7.22^{\text{ a}}$ | | |
| February | 79.11 ± 4.74 ^{cd} | $3.11 \pm 0.50^{\text{ cd}}$ | 24.96 ± 3.58 bc | | |
| March | 99.88 ± 4.23^{ab} | $3.83 \pm 0.42~^{abcd}$ | 29.6 ± 2.66 bc | | |
| April | 108.83 ± 4.69^{a} | $3.66 \pm 0.37~^{abcd}$ | 28.56 ± 3.29 bc | | |
| May | 94.72 ± 5.14 abc | 1.66 ± 0.39 cd | 33.1 ± 3.72^{b} | | |
| June | 93.5 ± 6.33 bc | 3.55 ± 0.37 bcd | 35.1 ± 3.41 ab | | |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI

Table 6. 5: Adult Neochetina weevils occurrence at Okana, 2001.

| | Numbe | er by species | |
|--------------|-----------------|------------------|-------|
| Month | N. bruchi | N. eichhorniae | Total |
| July 2001 | 25 ^a | 66 ^{ab} | 91 |
| August | 29 ^a | 61 abc | 90 |
| September | 27 ^a | 65 ^{ab} | 92 |
| October | 11 ^b | 30 ^{cd} | 41 |
| November | 3 ^b | 18 ^d | 21 |
| December | 5 ^b | 11 ^d | 16 |
| January 2002 | 12 ^b | 43 abcd | 55 |
| February | 4 ^b | 50 abcd | 54 |
| March | 11 ^b | 46 abc | 57 |
| April | 4 ^b | 33 bcd | 37 |
| May | 4 ^b | 71 ^a | 75 |
| June | 3 ^b | 45 abcd | 48 |
| Total | 138 | 539 | 677 |
| % Occurrence | 20.4 | 79.6 | |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI $\,$

Table 6. 6: Nature and extent of damage by *Neochetina* weevils on water hyacinth given by mean feeding scars and damaged petioles at Otho, 2001

| Month | Feeding Scars | Damaged Petioles | | | |
|--------------|--------------------------------|-------------------------|--------------------------------|--|--|
| | $(No.) \pm SE$ | Number ± SE | % damaged ± SE | | |
| July 2001 | 43.5 ± 3.79 ^g | 2.6 ± 0.64 ^a | 21.6 ± 4.56 ^{cd} | | |
| August | $48.27 \pm 6.24^{\ fg}$ | $1.94\pm0.48^{\ b}$ | 16.89 ± 3.96^{d} | | |
| September | 93.11 ± 7.91 abc | 2.61 ± 0.36^{ab} | 21.04 ± 2.95 cd | | |
| October | 75.55 ± 7.93 cde | 1.72 ± 0.33 b | 15.80 ± 2.88 d | | |
| November | 76.05 ± 6.62 bcde | $1.77\pm0.35^{\ b}$ | 18.08 ± 3.81^{d} | | |
| December | $66.88 \pm 4.03^{\text{ def}}$ | 2.55 ± 0.45 ab | 30.05 ± 5.11 abc | | |
| January 2002 | 53.55 ± 5.76 fg | 3.27 ± 0.49^{a} | 36.52 ± 5.62^{a} | | |
| February | 62.3 ± 5.33 ef | 2.16 ± 0.23 b | 21.77 ± 1.93 cd | | |
| March | 88.0 ± 8.11 abcde | $2.38\pm0.38~^{ab}$ | $24.61 \pm 4.0^{\ bcd}$ | | |
| April | 98.5 ± 10.11 abcd | 2.05 ± 0.22^{ab} | $24.9 \pm 2.4^{~ab}$ | | |
| May | 85.1 ± 8.13^{a} | $2.72\pm0.27^{\ b}$ | 32.93 ± 3.66 bcd | | |
| June | 94.94 ± 7.66 ab | $1.94\pm0.20^{\ b}$ | 21.96 ± 2.12 ^{cd} | | |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI

There were very few adult *N. bruchi* observed at Otho throughout the experimental period and no adults were recorded in the months of January to March and July 2002 (Table 6.7). No adults were also observed in November 2001. For *N. eichhorniae*, there were significant differences in the number of adults with June/July of 2002 having the highest numbers (80 and 75 respectively). The lowest numbers were counted in August 2001 (23), February 2002 (18) and April 2003 (16). In this case, as well, there was no clear pattern on the abundance of the weevils with time.

These results however generally suggested an empirical relationship either to environmental conditions or to the number of different stages of the two weevil species. The most destructive part of the life cycle of the weevils is the larval stage and a correlation analysis was carried out on a monthly basis to determine the relationships.

Table 6.8 shows the Pearson's Correlation Coefficient at Okana between the larval stage and various damages inflicted on the water hyacinth plant during the study period. From these relationships, there was apparent seasonality in the intensity of damage caused by the larvae in the months of August/October and March/April – June/July. These months correspond to the peak rainy seasons in the region.

The Pearson's correlation coefficient between the feeding scars and the number of larvae observed was insignificant throughout the study period.

Table 6.9 shows the Pearson's Correlation Coefficient at Otho between the most destructive part of the weevils life cycle (larvae) and various damages inflicted on the water hyacinth plant during the study period.

Table 6. 7: Adult Neochetina weevils occurrence at Otho, 2001.

| Month | Number | s by Species | Total |
|--------------|----------------|------------------|-------|
| Month | N. bruchi | N. eichhorniae | Total |
| August | 1 ^a | 23 ^a | 24 |
| September | 1 ^a | 37 ^b | 38 |
| October | 1 ^a | 30 ^a | 31 |
| November | 0 | 29 ^a | 29 |
| December | 1 ^a | 44 ^{cb} | 45 |
| January 2002 | 0 | 33^{d} | 33 |
| February | 0 | 16 ^a | 16 |
| March | 0 | 40 ^b | 40 |
| April | 1 ^a | 18 ^a | 19 |
| May | 1 ^a | 45 ^{cb} | 46 |
| June | 0 | 80 ^d | 80 |
| July | 0 | 75 ^d | 75 |
| Total | 6 | 470 | 476 |
| % Occurrence | 1.26 | 98.74 | |

Values with similar superscripts are not significantly different from each other at $95\%\,CI$

From these relationships, there was apparent seasonality in the intensity of damage caused by the larvae in the months of August/September and March/April – July. These months also correspond to the peak rainy seasons in the region.

For Otho as well, the Pearson's correlation coefficient between the feeding scars and the number of larvae observed was insignificant throughout the study period.

Table 6. 8: Monthly variation in Pearson's Correlation Coefficient and the corresponding p-values

| | Pearsons Correlation Coefficient | | | | n volue | | |
|-----------|---|----------|-----------|---------------|----------|-----------|--|
| | (Okana) | | | p-value | | | |
| | Feeding | Damaged | % Damaged | Feeding Scars | Damaged | % Damaged | |
| | Scars | Petioles | Petioles | recuing scars | Petioles | Petioles | |
| August | 0.359 | 0.583 | 0.609 | 0.144 | 0.011 | 0.007 | |
| September | -0.100 | -0.250 | -0.304 | 0.693 | 0.317 | 0.220 | |
| October | 0.165 | 0.610 | 0.326 | 0.514 | 0.007 | 0.186 | |
| November | -0.302 | 0.748 | 0.376 | 0.223 | 0.000 | 0.124 | |
| December | 0.433 | 0.011 | -0.068 | 0.073 | 0.965 | 0.788 | |
| January | 0.077 | 0.320 | 0.292 | 0.761 | 0.196 | 0.239 | |
| February | 0.087 | 0.288 | 0.317 | 0.730 | 0.246 | 0.199 | |
| March | 0.119 | 0.653 | 0.541 | 0.639 | 0.003 | 0.020 | |
| April | 0.251 | 0.412 | 0.474 | 0.316 | 0.089 | 0.047 | |
| May | -0.126 | 0.466 | 0.243 | 0.618 | 0.051 | 0.331 | |
| June | 0.057 | 0.534 | 0.463 | 0.822 | 0.022 | 0.053 | |
| July | 0.046 | 0.728 | 0.776 | 0.856 | 0.001 | 0.000 | |

Table 6. 9: Extent of damage and number of *Neochetina* weevils on water hyacinth given by mean feeding scars and damaged petioles at Otho, 2001

| • | Pearsons | S Correlation | n Coefficient | | n volue | |
|-----------|----------|-----------------|-----------------|---------|-----------------|-----------------|
| | (Otho) | | | p-value | | |
| | Feeding | Damaged | % Damaged | Feeding | Damaged | % Damaged |
| | Scars | Petioles | Petioles | Scars | Petioles | Petioles |
| August | -0.049 | 0.643 | 0.726 | 0.847 | 0.004 | 0.001 |
| September | -0.247 | 0.733 | 0.619 | 0.323 | 0.001 | 0.006 |
| October | 0.319 | 0.315 | 0.101 | 0.197 | 0.202 | 0.691 |
| November | -0.143 | 0.075 | 0.095 | 0.571 | 0.769 | 0.708 |
| December | -0.461 | 0.131 | 0.040 | 0.054 | 0.605 | 0.875 |
| January | 0.056 | 0.339 | 0.206 | 0.826 | 0.169 | 0.413 |
| February | 0.308 | 0.117 | 0.005 | 0.214 | 0.643 | 0.984 |
| March | -0.060 | 0.372 | 0.470 | 0.813 | 0.128 | 0.049 |
| April | 0.415 | 0.611 | 0.538 | 0.087 | 0.007 | 0.021 |
| May | -0.067 | 0.076 | 0.084 | 0.793 | 0.764 | 0.741 |
| June | -0.181 | 0.118 | 0.118 | 0.473 | 0.642 | 0.641 |
| July | -0.065 | 0.811 | 0.752 | 0.798 | 0.000 | 0.000 |

6.5.3 Correlation between plant parameters

To evaluate the correlation between selected plant parameters, Okana study site was selected as a representative site. A correlation analysis was done and the following was noted:

The number of feeding scars was related to the total number of petioles in the log scale (r=0.939). Figure 6.1 shows the relationship between the Log number of petioles with Log number of feeding scars. The relationship was significant (p<0.0005).

There was also a relationship between Log number of feeding scars and the Log laminar area (Figure 6.2). The correlation coefficient was 0.971 with p<0.0005.

The correlation between Log number of feeding scars and Log petiole length was 0.964 and p<0.0005 (Figure 6.3).

There was also a good correlation between the Log number of feeding scars and the Log number of adult *Neochetina eichhorniae* with r = 0.670 (Figure 6.4) but the relationship with *N. bruchi* was unexpectedly negative (Figure 6.5) with r = -0.819. In both cases, corresponding p-value was less than 0.0005.

The correlation between the Log number of damaged petioles and the Log number of petioles was positive with r = 0.767 and p<0.0005 (Figure 6.6).

There was also a significant correlation between the Log number of damaged petioles and Log laminar area (r = 0.754 and p<0.0005) as shown in Figure 6.7).

The correlation between the Log number of damaged petioles and Log petiole length showed that r = 0.726 (Figure 6.8). This relationship was also significant at 95% CI (p<0.0005).

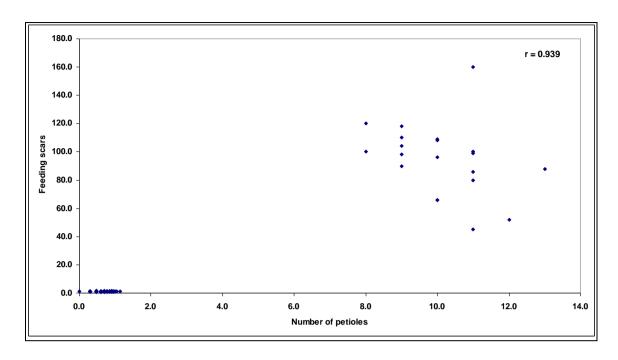


Figure 6. 1: Correlation between number of Petioles and Feeding scars at Okana, 2001

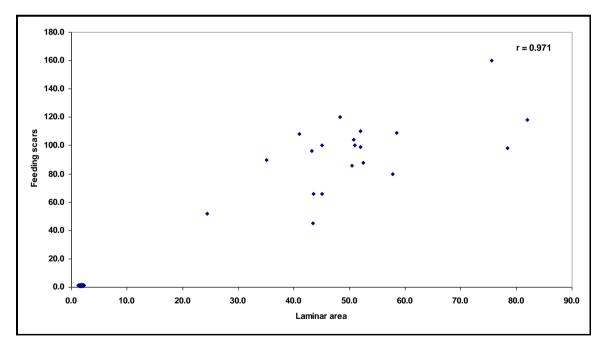


Figure 6. 2: Correlation between Laminar area and number of Feeding scars at Okana, 2001

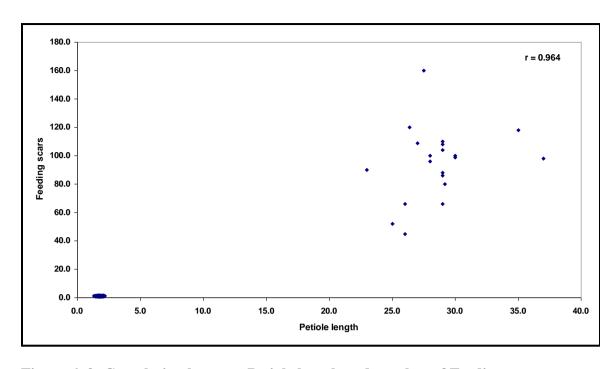


Figure 6. 3: Correlation between Petiole length and number of Feeding scars at Okana, 2001

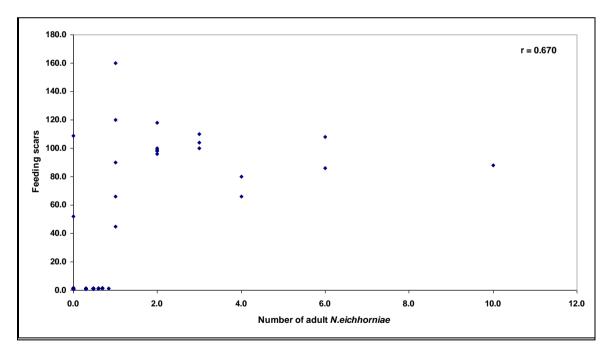


Figure 6. 4: Correlation between Adult *Neochetina eichhorniae* and number of Feeding scars at Okana, 2001

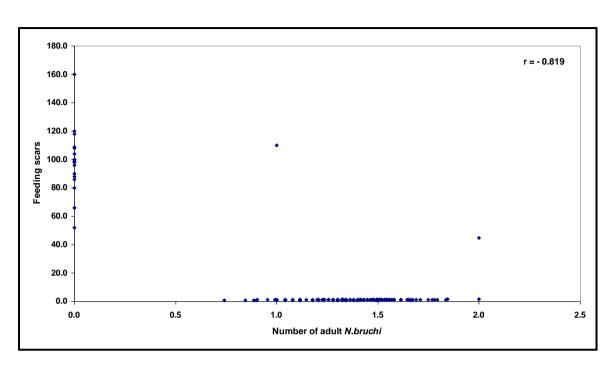


Figure 6. 5: Correlation between Adult *Neochetina bruchi* and number of Feeding scars at Okana, 2001

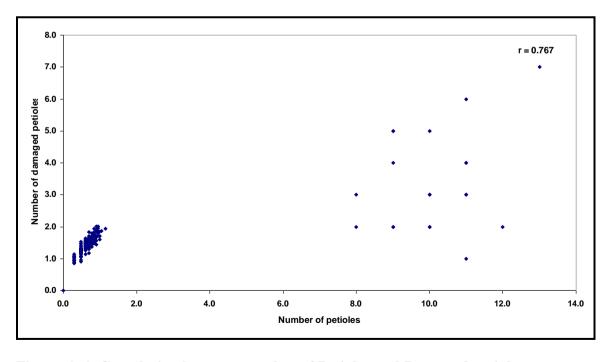


Figure 6. 6: Correlation between number of Petioles and Damaged petioles at Okana, 2001

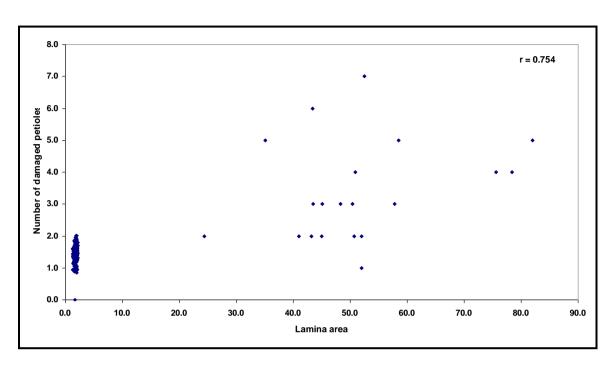


Figure 6. 7: Correlation between Laminar area and number of Damaged petioles at Okana, 2001

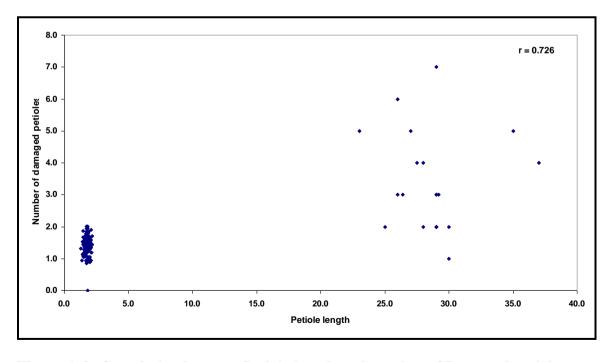


Figure 6. 8: Correlation between Petiole length and number of Damaged petioles at Okana, 2001

6.6 Discussion

For all the study sites, it was expected that the presence of *N. eichhorniae* and *N. bruchi* would result into greater damage to water hyacinth plants, their deterioration in health and eventual death. The process is also generally expected to result in effective biological control of the weed. This work relied on the premise that biological control in a semi-controlled environment can be replicated in the wild, to provide a scientific basis for control of water hyacinth using the *Neochetina* weevils. It is noteworthy that *N. eichhorniae* recorded a consistently higher proportion than *N. bruchi* in all the study sites.

The parameters that are considered in this study might not all be good indicators of the impact of the two weevil species on water hyacinth. However, there are strong indications from the result that the number of rametes observed, feeding scars and damaged petioles in both Okana and Otho reflect the possible impacts of the two weevil species on water hyacinth.

Close observations on the Pearson's correlation coefficient however revealed that there was no significant relationship between the larval stages of the weevils and feeding scars but there was a significant relationship between the larvae of both species of the weevil and damaged petioles. It therefore follows that the number of damaged petioles is a good indicator of the impact of the weevils on water hyacinth. The importance of the larval stage in the control of water hyacinth in the Lake Victoria Basin is thus corroborated.

As observed in Budalangi, there were significant differences in many of the parameters between dates. This is probably an indication of the varying environmental conditions during the study period. It is noteworthy to state that the pond at Budalangi dried up completely during the study period and data could only be obtained for six months. The long-term impact of the weevils on water hyacinth could not be investigated due to these environmental conditions which caused rapid deterioration and wilting of water hyacinth at this site. It was thus difficult to determine the fate of the weevils during the study period. Thus, such environmental changes could interfere with both the host weed and the biological control agent.

At Otho and Okana sites, with permanent water bodies the parameters showed differences between sampling dates in most cases as well. It is apparent that the way the transects were taken in a pond varied from one end to the next in terms of depth and other parameters. Since water hyacinth is a floating weed, shallow depths along the transects are likely to interfere with growth and survival of the weed.

Judging from results found at Okana, the most significant impacts could be observed on the number of rametes, and damaged petioles. A good indicator for impact is the number of damaged petioles (Julien *et. al*, 1999). However, there was a corresponding difference in the numbers of larvae, and adults of the weevils. Damage to the weed could also be attributed to the increase in number of larvae and adults during the study period at Okana.

It may therefore be justified to state that the impact observed on water hyacinth is attributed to the increase in density of egg laying, larvae and growth to adult stages of the two weevil species. The larva causes critical damage to the petioles while the adults cause damage by feeding on the leaves. Harsh environmental conditions only seem to help in speeding up the process of damage to the plants.

It is also possible to consider the petiole length and laminar area while assessing the impact of the two weevils on water hyacinth. Since significant differences of these parameters was apparent between transects, it can be suggested that the impact is likely to be influenced by hydrographic conditions in the pond and environmental conditions in addition to the weevils themselves.

Pearson's Correlation Coefficient indicates that there was a good relationship between the damage caused. Such damage parameters included the feeding scars, damaged petioles and percentage of damaged petioles in some specific months during the study period. These specific months correspond to the peak rainfall periods established for the region. Rainfall patterns are definitely linked to daily temperature regimes, humidity and wind patterns in the region. High correlation is therefore also likely to be a reflection of the prevailing environmental conditions that enhance the rapid production of larvae, which is the most destructive stage of these insects.

Similarly at Otho site, the numbers of larvae were more objectively related to the number of damaged petioles. This is an expected trend since the larvae tunnel into the petiole of the plant where it matures. Feeding scars were also found to be more objectively related to the number of adults observed. This means that as the number of adults increased, the number of feeding scars also increased. Adult weevils feed on the leaves and besides reducing the photosynthetic area, the damaged leaves provide

good sites for opportunistic fungi. The latter further weakens the plants and contributes to its eventual death.

It can be concluded that the petioles are more prone to damage as the number of larvae increases and at the same time, the feeding scars will correspondingly increase as more larvae mature into pupae and eventually into adults. Biological control is based on the ability of the control agent to inhibit efficient growth, reproduction and performance by the host. In the case of water hyacinth and the *Neochetina* weevils the process may be slow but effective results can only be achieved with time. As the results indicate, differences in time for different indicators of impact are a pointer in the importance of time as a secondary control factor. In such a case, it might be better to reduce the time factor by increasing the density of weevils being used for control of the weed. It might be necessary to determine how long a given density of the weevil will take to kill a healthy water hyacinth plant in the natural environment. Julien *et al.*, (1999) have estimated this threshold at 5 weevils per plant.

CHAPTER SEVEN

7.0 General Discussion and Recommendations

7.1 General Discussion

From the results of life cycle and fecundity studies, there is a clear indication that *Neochetina bruchi* lays more eggs than *N. eichhorniae*. Even though these differences existed, there is no evidence that the larval density of both species is not equally high enough for use as effective biological control agents for water hyacinth. Indeed, the synergistic effects of the two species are relatively un-investigated. It would therefore be interesting to evaluate the impact of isolated single weevil species on the water hyacinth. This was, however, not one of the objectives of this study. Some caution need to be exercised in using regression to predict the amount of eggs laid for each of the weevil species since only 54.4% of the regression could be explained by the data for *N. bruchi* and only 45.3% of the regression could be explained by data for *N. eichhorniae*. In both cases, it was however very clear that egg production for both species declines with time in weeks.

A significant relationship was also found between fecundity and temperature but there was low fit in the data with only 37.8% and 28.7% of the data being explained in *N. bruchi* and *N. eichhorniae* respectively. The low fit of data again shows that the information should be used cautiously unless additional data is available for verification.

The general trend showed that the egg to larva stage took between 7.6 to 11 days while that of larvae to pupa took 31 to 35 days. The pupal stage took between 30 and 33 days. There seem to be a general agreement in the generation time of the weevils from various studies (Ogwang and Molo, 1997; DeLoach and Cordo, 1976). It is therefore possible to derive a time dependent population model for the weevil and compare it with a similar model for water hyacinth. This approach can give fairly accurate estimates of the number of weevils required to control a given biomass of water hyacinth in a given period. During the study period, no suitable model could be found to simultaneously address the issue.

The method used for incubating the eggs in the laboratory determines the survival rate of the weevils. Survival rates of 33.8% for incision egg setting is definitely higher than 18.7% for free egg setting for *N. bruchi*. The technique that resembles the natural behaviour of the weevil as close as possible is the IES where, the larvae on emergence, starts tunneling through the plants stem. *N. eichhorniae* on the other hand had even lower survival rate at 25.4% for IES and 19.7% for FES. These observations are direct indicator that the eggs must be laid inside the plants for high survivorship. At the same time, it shows that there are significant differences in the survival of each species of the weevil. However, the recommendation of using only one species for biological control is still not justifiable in the absence of conclusive evidence on the exclusive impact of each species and synergistic effects.

The results clearly indicate that the survival rate increases from egg to adult stage. The most critical stage for biological control of water hyacinth is the larval stage. It is therefore important that strategies in the natural environment that enhance larval

survival are adopted. One such approach may involve inoculating the plants with laboratory-reared larvae and at high enough densities to cause enough damage to water hyacinth to produce the desired effect. Ochiel and Njoka (2001) have shown that releasing infested plants onto mats of water hyacinth enhances the mats deterioration.

It is clear from the results that there were seasonal variations in the extent of damage caused to the water hyacinth plants by the weevils. Highest mean number of rametes seemed to be inversely related with the petiole length and was observed in July for all the study sites. This is an indication of similar morphological characteristics of the water hyacinth plant. This observation implies that similar approaches to biological control can be applied equally and similarly at all sites.

At all the study sites, the longest petioles and largest laminar areas were recorded from March to May. In both Okana and Otho sites, the highest number of total petioles was highest in the months of August and September which also showed relatively higher number of feeding scars. This is an indicator of upsurge in feeding by adults during the faster and profuse growth of the water hyacinth. The situation is ideal for effective biological control of the weed. The growth should indeed be checked during this period and if there are indications that growth and attacks are more during the same period, then biological control initiative is likely to be more effective. The number and percentage of damaged petioles reflects more activity of the weevils from January onwards. However, it is not possible to clearly relate the amount of damage inflicted on the water hyacinth with density of the adult weevils.

This situation is also reflected in the lack of clear pattern of weevil abundance with time.

Correlation analysis revealed suspicions that the extent of damage could be related to either the environmental conditions or to the density of weevils for some months on each site. There is indication that strong correlation between larvae and feeding scars, number of damaged petioles and percentage of damaged petioles shows that the impact of the weevil species is derived from the larval stage. However, the feeding intensity, judged from the feeding scars seems to follow some undefined seasonal pattern. It is probably possible to follow the seasonal pattern if adequate data on environmental conditions in the field are available. Under semi-artificial environment, it is not possible to make such clear distinctions.

7. 2 Conclusions

From these studies the following conclusions were made:

- The Neochetina weevils are effective biological control agents of water hyacinth in the Lake Victoria Basin, Kenya.
- 2. The two weevil species have a significantly different egg laying capacity with the *Neochetina bruchi* laying more eggs than *N. eichhorniae*.
- 3. The egg to larva survival rate of the two insect species was not significantly different. This makes both species equally good biological control candidates since the larva is the critical stage in destroying the plant.
- 4. The ambient weather conditions do not affect the development of the weevils.

7.3 **Recommendations**

- Since biological control is a relatively alien concept, communities
 living around the Lake Basin should be trained on the weevil mass
 rearing and subsequent release techniques.
- 2. The use of weevils can be supplemented with other control strategies e.g mycoherbicides, physical control as well as indirect methods such as the observance of good agricultural and industrial practices which reduce pollution loading.
- 3. The release of the weevils should be extended to river mouths emptying into Lake Victoria. These have been water hyacinth "hotspots" since the weed thrives well in the nutrient loaded lake entry sites.
- 4. Since River Kagera is a major source of water hyacinth introduction into Lake Victoria, collaborative biological control programmes should be extended to cover the entire basin. Better still, Rwanda and Burundi should be encouraged to join the East African Community.
- 5. Since these are pioneer studies in East Africa, it is recommended that further investigations be done to establish *inter alia:*
 - a) The threshold number of weevils required to subdue a known weed area.
 - b) The possible synergistic effect of these weevils and known strains of mycoherbicides in combating water hyacinth.

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 $\label{eq:annexES} \textbf{ANNEXES}$ Annex I: Mean scores (± SE) for plant parameters at Okana, 2001.

| Month | Rametes (No.) ± SE | Petiole Length (cm) ± SE | Laminar Area (cm²)± SE | Total Petioles (No.) ± SE |
|---------------|--------------------------------|-----------------------------|---------------------------|------------------------------|
| July, 2001 | 2.16 ± 0.23^{ab} | 25.98 ± 1.46 cde | 54.7 ± 4.81 bc | 14.22 ± 0.66 ab |
| August | 1.88 ± 0.24 bc | 24.19 ± 1.06^{def} | 54.61 ± 3.91 bcd | 16.05 ± 1.22^{a} |
| September | $2.16\pm0.18~^{ab}$ | $22.58\pm1.07^{\rm \ f}$ | 54.37 ± 3.03 bcd | 14.11 ± 0.59 abc |
| October | 2.44 ± 0.21^{a} | 28.38 ± 0.95 abc | 98.56 ± 6.91 ^a | 11.77 ± 0.73 cde |
| November | $2.55\pm0.24~^{a}$ | 27.2 ± 1.06 bcd | 56.88 ± 3.64 bc | 13.11 ± 0.97 bcd |
| December | $2.16\pm0.25~^{ab}$ | 23.96 ± 1.15 ef | 46.15 ± 3.82 cd | 13.5 ±1.24 bc |
| January, 2002 | 1.33 ± 0.11^{d} | $28.04 \pm 0.81~^{abc}$ | $58.13 \pm 1.87^{\ b}$ | 9.61 ± 0.42 |
| February | $1.55 \pm 0.20^{\ c\ d}$ | 26.1 ± 0.96 bcde | 44.37 ± 2.53 d | 12.66 ± 0.87 bcd |
| March | 1.35 ± 0.11 ^{c d} | 30.1 ± 1.95 ab | 54.35 ± 2.84 bcd | 12.83 ± 0.80 bcd |
| April | 1.1 ± 0.07 d | 45.6 ± 0.81 | 94.1 ± 4.46^{a} | $13.2\pm0.87^{\ bcd}$ |
| May | 1.27 ± 0.13^{d} | 30.5 ± 1.07^{a} | 51.1 ± 3.46 bcd | 10.94 ± 0.78 de |
| June | 1.05 ± 0.05 d | 28.56 ± 0.77 abc | 52.1 ± 3.43 bcd | 10.16± 0.31 ^e |

Values in the same column with similar superscripts are not significantly different from each other at 95% CI.

Annex II: Mean scores $(\pm SE)$ for plant parameters at Otho, 2001.

| Month | Rametes (No.) ± SE | Petiole Length (cm) ± SE | Laminar Area (cm²)± SE | Total Petioles (No.) ± SE |
|--------------|-----------------------------|--------------------------------|--------------------------------|-------------------------------|
| July 2001 | 1.77 ± 0.22 ^a | 23.5 ±1.38 ^{cde} | 49.37 ±3.88 ^{cd} | 11.1 ± 0.62 bc |
| August | $1.94\pm0.17^{\rm \ a}$ | 20.56 ± 1.33^{e} | 39.47 ± 3.84^{d} | $11.77\pm0.86~^{ab}$ |
| September | $1.72\pm0.13^{\ a}$ | 25.58 ± 1.19 cd | $51.96 \pm 3.60^{\text{ c}}$ | 12.72 ± 0.54^{a} |
| October | $1.16 \pm 0.12^{\text{ c}}$ | 25.42 ± 0.81 cd | 49.85 ± 2.93 ^{cd} | 11.11 ± 0.85 bc |
| November | 1.61 ± 0.23^{ab} | $22.97 \pm 1.07^{\text{ de}}$ | 53.52 ± 4.19 bc | 10.5 ± 0.60 cd |
| December | $1.11 \pm 0.07^{\text{ c}}$ | 26.62 ± 1.39 bc | 52.22 ± 3.41 ° | 8.33 ± 0.49 ef |
| January 2002 | $1.16 \pm 0.12^{\text{ c}}$ | $30.0\pm1.45~^{ab}$ | 50.13 ± 2.77 cd | 8.94 ± 0.41^{def} |
| February | $1.16 \pm 0.09^{\text{ c}}$ | 23.57 ± 1.65 cde | 41.23 ± 3.43 cd | 9.83 ± 0.51 cde |
| March | $1.22 \pm 0.12^{\text{ c}}$ | 36.22 ± 1.41 | 66.75 ± 4.41 ab | 9.61 ± 0.24 cde |
| April | 1.11 ± 0.07 bc | 32.12 ± 2.02 cd | 69.04 ± 7.67 cd | $8.05 \pm 0.31^{\text{ def}}$ |
| May | $1.22 \pm 0.12^{\text{ c}}$ | 24.53 ± 1.36 ^a | 44.41 ± 2.77 ^a | $8.5 \pm 0.39^{\text{ f}}$ |
| June | 1.22 ± 0.15 bc | 26.13 ± 1.44 ^{cd} | 53.41 ± 4.59 bc | 8.88 ± 0.38 def |

Values in the same column with similar superscripts are not significantly different from each other at $95\%\ CI$