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A MODEL FOR WATER HYACINTH BIOLOGICAL CONTROL

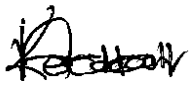
Kendall Adair Hauptfleisch

A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science

The financial assistance of the National Research Foundation (DAAD-N RF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the DAAD-N RF.

DECLARATION

I, Kendall Adair Hauptfleisch, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



_____

Signature of the candidate

_____01_____ day of _____July___ in 2015

ABSTRACT

Water hyacinth is one of the most invasive aquatic plants in the world. As such, there have been numerous attempts to model and predict its growth. Some of these models incorporate the influence of temperature or nutrients as the two most important determinants of water hyacinth growth. Other models include the effect of biological control on the growth of the plant, but only one model integrates environmental factors (temperature) with the effect of biological control. In this study, I attempt to incorporate temperature, and biological control effects on the growth of water hyacinth into a single model. Temperature-dependent water hyacinth and stage-structured *Neochetina* weevil population models were constructed in STELLA 9.1.4 and compared against an empirical dataset for two water hyacinth infested sites in South Africa for a two-year period (2004-2006). Although these models may not simulate field water hyacinth populations accurately, they suggest that *Neochetina* weevils can reduce water hyacinth populations, to below the assumed carrying capacity (70 kg/m^2). It appears that the effects of *Neochetina* larvae are vital in reducing water hyacinth populations, and need to be further explored in order to simulate water hyacinth/weevil systems accurately.

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TABLE OF CONTENTS

CONTENTS **PAGE**

DECLARATION ..... **II**

ABSTRACT ..... **III**

ACKNOWLEDGEMENTS ..... **IV**

LIST OF FIGURES ..... **VIII**

LIST OF TABLES ..... **XIII**

1 CHAPTER ONE – INTRODUCTION **1**

1.1 Rationale 1

1.2 Objectives 2

1.3 Literature Review 3

1.3.1 Water hyacinth, *Eichhornia crassipes* 3

1.3.2 *Neochetina* water hyacinth weevils 9

1.3.3 Modelling biological control systems 12

1.3.4 The way forward 22

1.3.5 Structure of the dissertation 23

2 CHAPTER TWO – TEMPERATURE-DEPENDENT DEVELOPMENT AND FEEDING OF *NEOCHETINA EICHHORNIAE* **24**

2.1 Introduction 24

2.1.1 Temperature and insect development 24

2.1.2 Line-fitting methods for the estimation of degree-day thresholds 25

2.1.3 Temperature and insect herbivory 26

2.1.4 Objectives 28

2.2 Methods 28

2.2.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs 28

2.2.2 Estimating feeding damage by *Neochetina eichhorniae* larvae 29

2.2.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults 30
2.3 Results 31
2.3.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs 31
2.3.2 Estimating feeding damage by *Neochetina eichhorniae* larvae 33
2.3.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults 35
2.4 Discussion 38
2.4.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs 38
2.4.2 Estimating feeding damage by *Neochetina eichhorniae* larvae 40
2.4.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults 42
2.5 Conclusion 46
3 CHAPTER THREE – CONSTRUCTING A MODEL OF WATER HYACINTH BIOLOGICAL CONTROL 47
3.1 Introduction 47
3.1.1 Types and uses of models in biological control 47
3.1.2 Model population dynamics and parameterisation 49
3.2 Methods 50
3.2.1 Model construction 50
3.2.2 Modelling water hyacinth 52
3.2.3 Modelling *Neochetina eichhorniae* populations 55
3.2.4 Combined water hyacinth biocontrol models 72
4 CHAPTER FOUR – SIMULATION AND VALIDATION OF THE MODEL OF WATER HYACINTH BIOLOGICAL CONTROL 77
4.1 Introduction 77
4.2 Methods 79

4.3 Results 80
4.3.1 Model simulation 80
4.3.2 Model validation 93
4.4 Discussion 96
4.4.1 Water hyacinth models 96
4.4.2 *Neochetina eichhorniae* models 97
4.4.3 Combined models of water hyacinth biocontrol 100
4.5 Conclusion 106
5 CHAPTER FIVE – GENERAL DISCUSSION 107
5.1 Modelling systems 107
5.1.1 Food for thought 109
5.1.2 The ideal model 111
5.2 Conclusion 113
6 APPENDIX 115
7 REFERENCES 122

LIST OF FIGURES

Figure 1.1: Global distribution of water hyacinth (data compiled from GBIF, EDDMapS, and Rhodes, Department of Zoology and Entomology from Smit, 2013)..... 3

Figure 1.2: A generalized sketch of water hyacinth plants showing the growth form which occurs in A. dense mats as compared to the growth form that occurs in B. more open situations (After Center and Spencer, 1981)..... 5

Figure 1.3: Logistic (dashed red) versus exponential (solid blue) growth population models..... 15

Figure 1.4: Wilson *et al.,* (2001) models of water hyacinth and weevil populations A. assumes all weevil stages have the same effect on the plant and B. includes a time delay to mimic larval damage to water hyacinth populations..... 20

Figure 1.5: Monthly water hyacinth biomass at Delta Park, South Africa (After Byrne *et al.,* 2010). Compare the weed population over time with that in Figure 1.4 and note that the water hyacinth population does not go extinct as in Figure 1.4..... 21

Figure 2.1: The effect of temperature on development of *Neochetina eichhorniae* eggs using the reduced major axis regression, where DT is the product of the egg duration and temperature (n=101; Ikemoto and Takai, 2000). The solid line represents the egg major axis regression $DT = t_E.D + K_E$. 32

Figure 2.2: The effect of temperature on the survival of *Neochetina* e*ichhorniae* eggs (n=225). The solid line represents the second order polynomial regression between 15-30°C. Temperatures where no hatches occurred were not included in the regression. 33

Figure 2.3: Effect of larval feeding by *Neochetina eichhorniae* on water hyacinth plant biomass (wet weight, g) at 25°C over 51 days. Plants were grown in 2.8 mg/L N and 0.4 mg/L P nutrient solutions at 25°C and were destructively sampled each week (n=2-8 per treatment per week, means ± SD). Plant biomass each week was compared to the initial biomass..... 34

Figure 2.4: Biomass removal by *Neochetina eichhornaie* larvae over 51 days using estimates of larval numbers. Plants were grown in 2.80 mg/L N and 0.40 mg/L P nutrient solutions at 25°C. The line A indicates a mean of 0.90 g/larva/day between day 15 and day 51. 34

Figure 2.5: The effect of temperature on *Neochetina eichhorniae* adult feeding rates (means ± SD). Different nutrient and leaf age treatments have been combined for each temperature treatment. Significant differences between means are indicated by different letters. 35

Figure 2.6: The effect of leaf age on *Neochetina eichhorniae* adult feeding rates (means ± SD). Lower leaf numbers correspond to younger leaves. Nutrient and temperature treatments were combined per leaf. Different temperature treatments have been combined for each leaf age treatment.

Significant differences between means are indicated by different letters. 36
Figure 2.7: The effect of nutrients on *Neochetina eichhorniae* adult feeding rates for plants grown at high [ca. 6.00 mg/L N; 0.86 mg/L P], medium [2.00 mg/L N; 0.29 mg/L P], and low [0.67 mg/L N; 0.10 mg/L P] nutrients at increasing temperatures (means ± SD). Different leaf numbers were combined to determine an overall temperature*nutrient interaction. 36
Figure 2.8: The effect of temperature on *Neochetina eichhorniae* adult feeding rates on water hyacinth plants grown at high [ca. 6.00 mg/L N; 0.86 mg/L P], medium [2.00 mg/L N; 0.29 mg/L P], and low [0.67 mg/L N; 0.10 mg/L P] nutrients (means ± SD). Both high and low nutrient values have been slightly offset for ease of graph interpretation. 37
Figure 3.1: Illustration of systems modelling notation in STELLA. Direction of flow is determined by the direction of the arrowhead. Positive flows are directed into the stock, while negative flows are directed away from the stock. 51
Figure 3.2: Stage 1 (excluding temperature) model of logistic water hyacinth growth in STELLA. The water hyacinth population density (V) is modified by the intrinsic population growth rate (r_v) (0.052 g/g/day; Center *et al.*, 1982) and the system carrying capacity (K) (70 kg/m^2; Reddy and D'Angelo, 1990). 53
Figure 3.3: Stage 2 (temperature) model of logistic water hyacinth population growth in STELLA. The relative growth rate of the population (R) is modified by variable water temperature (T_w) according to van der Heide *et al.*, (2006). New model elements are shown in solid orange while previous model elements that have been altered are crosshatched in orange. 54
Figure 3.4: Simple egg model. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with + function as sums of allocated arrays (egg density). 57
Figure 3.5: Simple degree-day model. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. 58
Figure 3.6: Stage 1 (constant oviposition) egg life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (egg density). New model elements are shown in solid orange. 60

Figure 3.7: Stage 1 (constant oviposition) larval life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (larval density). 61
Figure 3.8: Stage 1 (constant oviposition) pupal life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (pupal density). 63
Figure 3.9: Stage 1 (constant oviposition) adult life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed..... 64
Figure 3.10: Stage 2 (temperature-dependent) egg life stage model module. Stage 2 models assume temperature-dependent oviposition rates (O_r; DeLoach and Cordo, 1976). Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (egg density). Variables that have been altered from Stage 1 models are crosshatched in orange, while new elements are solid orange. 66
Figure 3.11: Stage 2 (temperature-dependent) larval life stage model module. Stage 2 models assume temperature-dependent egg survival proportions (S_E). Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (larval density). Variables that have been altered from Stage 1 models are crosshatched in orange. 67
Figure 3.12: Stage 3 (winter mortality) A. egg and B. larval life stage model modules. Stage 3 models assume temperature-dependent oviposition rates (O_r; DeLoach and Cordo, 1976) and winter mortality. Layered stocks, flows and converters are arrayed variables. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (A. egg density; B. larval density). New variables that have been introduced to Stage 2 models are solid orange..... 70

Figure 3.13: Stage 3 (winter mortality) adult life stage model module. Stage 3 models assume that adult weevils oviposit at maximum rates for only 21 days of the 104-day life span. Layered stocks, flows and converters are arrayed variables. Converters marked with + function as sums of allocated arrays (total old + young weevils). New variables that have been introduced to Stage 2 models are solid orange. 71
Figure 3.14: Stage 4 (integrated) model. Stage 4 models assume variable water and canopy temperatures (T_w, T_c), maximum area removed through adult herbivory (m) as well as variable maximum rate of larval herbivory (c_2). Converters marked with ~ can vary over time and retrieve data from a temperature graph. New model elements are indicated in solid orange. 74
Figure 4.1: Stage 1 (excluding temperature) logistic growth model of water hyacinth; V is the water hyacinth biomass density and r_V is the intrinsic growth rate of water hyacinth 0.052 g/g/day; starting biomass 12.4 kg/m^2. 80
Figure 4.2: Output from Stage 2 (temperature) model of water hyacinth growth under A. low variable water temperatures (Delta Park: Monthly temperature minimum and maximum 5.4°C and 23.8°C respectively) and B. high variable temperatures (Mbozambo Swamp: monthly temperature minimum and maximum 15.4°C and 32.9°C respectively); V is the water hyacinth biomass density and R is the relative growth rate of water hyacinth under fluctuating temperatures. The dotted line indicates the point of zero population growth. 81
Figure 4.3: Water hyacinth A. canopy temperature and B. water temperature over 2 years (2004-2006) at Delta Park (blue) and Mbozambo Swamp (red). Day 1 corresponds to 01 December 2004. 82
Figure 4.4: The effect of temperature on the oviposition of *Neochetina eichhorniae* at five constant temperatures (data from DeLoach and Cordo, 1976). The solid line represents the third order polynomial regression. 83
Figure 4.5: Modelled temperature-dependent oviposition (O_r) at Delta Park (blue) and Mbozambo Swamp (red) for Stage 2 (temperature-dependent) models. Day 0 corresponds to 01 December 2004. 83
Figure 4.6: Modelled temperature-dependent egg survival proportion (S_E) at Delta Park (blue) and Mbozambo Swamp (red) for Stage 2 (temperature-dependent) models. Day 0 corresponds to 01 December 2004. 84
Figure 4.7: Stage 2 (temperature-dependent) *Neochetina eichhorniae* model population densities (individuals/m^2) of egg (blue), larval (red), pupal (green) and weevil adult (purple) life stages for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site). Stage 2 models include temperature-dependent oviposition rates and egg survival proportions. The scale differs on the y-axes. 85

Figure 4.8: Stage 3 (winter mortality) *Neochetina eichhorniae* model population densities (individuals/m^2) of egg (blue), larval (red), pupal (green) and weevil adult (purple) life stages for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site). Stage 3 models include temperature-dependent oviposition rates and egg survival proportions, egg and larval winter mortality, and age-dependent oviposition. The scale differs on the y-axes. 86
Figure 4.9: Minimum water hyacinth canopy temperatures over 2 years (2004-2006) at Delta Park (blue) and Mbozambo Swamp (red) (Byrne *et al.*, 2010*)*. 87
Figure 4.10: The effect of temperature on *Neochetina* adult weevil feeding. Data that was expressed in scars was converted to mm^2 using a conversion of 4.5mm^2/scar. The solid line represents a third order polynomial regression of adult weevil feeding rate against temperature. 88
Figure 4.11: The effect of temperature on *Neochetina* larval weevil feeding. Larval feeding was measured at 25°C (red). All other temperatures were estimated using the adult temperature-dependent feeding relationship and a larval conversion factor (L_f) 89
Figure 4.12: Stage 4 (integrated) model population densities of water hyacinth (*V*) and *Neochetina* larval plant biomass removal (*Larval feeding*). Water hyacinth populations were modelled under temperature-driven feeding by *Neochetina eichhorniae* adults and larvae for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site)..... 90
Figure 4.13: Stage 4 (integrated) model – simulated *Neochetina* adult weevil plant biomass removal for Delta Park (blue) and Mbozambo Swamp (red). Compare with Figure 4.12 to see the difference in the scale of larval to adult plant biomass removal. 91
Figure 4.14: Stage 4*b* (larval carrying capacity) model population densities of *Neochetina* adults (purple) and larvae (red), water hyacinth (*V*, blue) and larval plant biomass removal (green). Weevil and water hyacinth populations were modelled for A & C. Delta Park (cold site) and B & D. Mbozambo Swamp (warm site). 92
Figure 4.15: Stage 4*b* (larval carrying capacity) model – simulated *Neochetina* adult weevil plant biomass removal at Delta Park (blue) and Mbozambo Swamp (red). 93
Figure 4.16: Comparison between Stage 4*b* (larval carrying capacity) modelled populations and field populations for Delta Park (A, C, E) and Mbozambo Swamp (B, D, F). Water hyacinth (blue), larval (red), and adult weevil simulated populations (purple) are shown by solid lines; all field populations are shown by dotted lines. * Significant interactions (source*season) ** Significant difference in means..... 95

LIST OF TABLES

Table 2.1: Approximate concentrations of macronutrients (as specified by Hoagland and Arnon, 1950) in high, medium, and low nutrient solutions used to culture water hyacinth for temperature/nutrient feeding experiments. 31
Table 2.2: The effect of temperature on the hatching time and % mortality of the *Neochetina eichhorniae* eggs at constant temperatures (n=225). 32
Table 3.1: Applications of modelling in biological control systems 47
Table 3.2: Lower developmental thresholds (*t*) and thermal constants (*K*) for immature stages of *Neochetina eichhorniae* using the reduced major axis regression. (Adapted from King, 2011) 56
Table 3.3: Oviposition rate by *Neochetina eichhorniae* at six constant temperatures (Taken from DeLoach and Cordo, 1976). 67
Table 3.4: Leaf area removed from water hyacinth leaves through adult *Neochetina eichhorniae* herbivory..... 73

1 CHAPTER ONE – INTRODUCTION

1.1 Rationale

Water hyacinth is one of the world's most invasive aquatic plants. Originating from South America, it has invaded many ecosystems worldwide and numerous water bodies in South Africa since its introduction in 1908 (Gopal, 1987). With the cost of controlling invasive alien plants in South Africa exceeding R6.5 billion per annum (van Wilgen and De Lange, 2011) and water hyacinth being considered as one of South Africa's worst weeds (Cilliers, 1991; Byrne *et al.,* 2010), control of water hyacinth in South African water systems is crucial.

Various methods such as mechanical, chemical and biological control have been used against this weed (Penfound and Earle, 1948; Gutiérrez *et al.,* 2001; Hill, 2003). However, classic biological control is considered to be more sustainable (MacFadyen, 1998; Hill, 2003; Hoelmer and Kirk, 2005), and is potentially 400 times more cost effective than herbicidal control, when successful (van Wyk and van Wilgen, 2002). With such an economic benefit, understanding and improving the success of biological control of water hyacinth is essential.

Modelling plant-herbivore systems can provide insight into understanding mechanisms underlying the success or failure of biocontrol efforts (Kriticos, 2003; Sheppard *et al.,* 2005; Holst *et al.,* 2007). Although several models of water hyacinth growth have been developed (Mitsch, 1976; Wilson *et al.,* 2005), most consider only single factors, such as herbivory (Wilson *et al.,* 2001), or the effect of individual nutrients (Reddy *et al.,* 1989), or temperature on water hyacinth growth, without integration of these elements. Consequently, understanding the combination of multiple drivers of plant growth under changing conditions is vital to determine management and control strategies for a pest as pervasive as water hyacinth.

Developing a mechanistic model of water hyacinth and its weevil populations with predictive capabilities will not only increase the understanding of the water

hyacinth/weevil systems and success of biological control, but also inform management decisions for control, both immediately and in the future. This may have extensive economic and environmental benefits.

This study therefore proposes to incorporate the effects of biological control by *Neochetina eichhorniae* weevils and temperature (on both weevils and plants) into a model of water hyacinth growth that will give site-specific predictions of population growth of both weevils and water hyacinth. Furthermore, as water hyacinth has more than one control agent released against it in South Africa, such as *Cornops aquaticum* (Bownes *et al.*, 2010*a*) and *Eccritotarsus catarinensis* (Ajuonu *et al.,* 2009), the success of this research may provide proof of principle for modelling both potential and current biological control agents of water hyacinth.

1.2 Objectives

The objectives of this research project are as follows:

1) Determine how environmental temperature affects water hyacinth growth and model this relationship.
2) Determine how environmental temperature affects *N. eichhorniae* development and feeding and model the relationship.
3) Develop a temperature-dependent, stage-structured population model for *N. eichhorniae* weevils.
4) Combine the *N. eichhorniae* and water hyacinth models to predict plant and weevil populations for specific water hyacinth infestations.

1.3 Literature Review

1.3.1 Water hyacinth, *Eichhornia crassipes*

Water hyacinth is a perennial, floating macrophyte first described by Von Martius in 1823 (Edwards and Musil, 1975). The plant is native to Brazil (Penfound and Earle, 1948) but has invaded over 70 countries across the globe (Figure 1.1; Julien *et al.*, 2001), becoming one of the world's most invasive aquatic plants. Water hyacinth was first recorded in South Africa in 1908 (Gopal, 1987), having been brought into Cape Town (Jacot Guillarmod, 1979) and Natal (Edwards and Musil, 1975) most likely as an ornamental plant (Jacot Guillarmod, 1979). However, it has since become a pest in many South African water systems (Cilliers 1990; Hill, 2003).

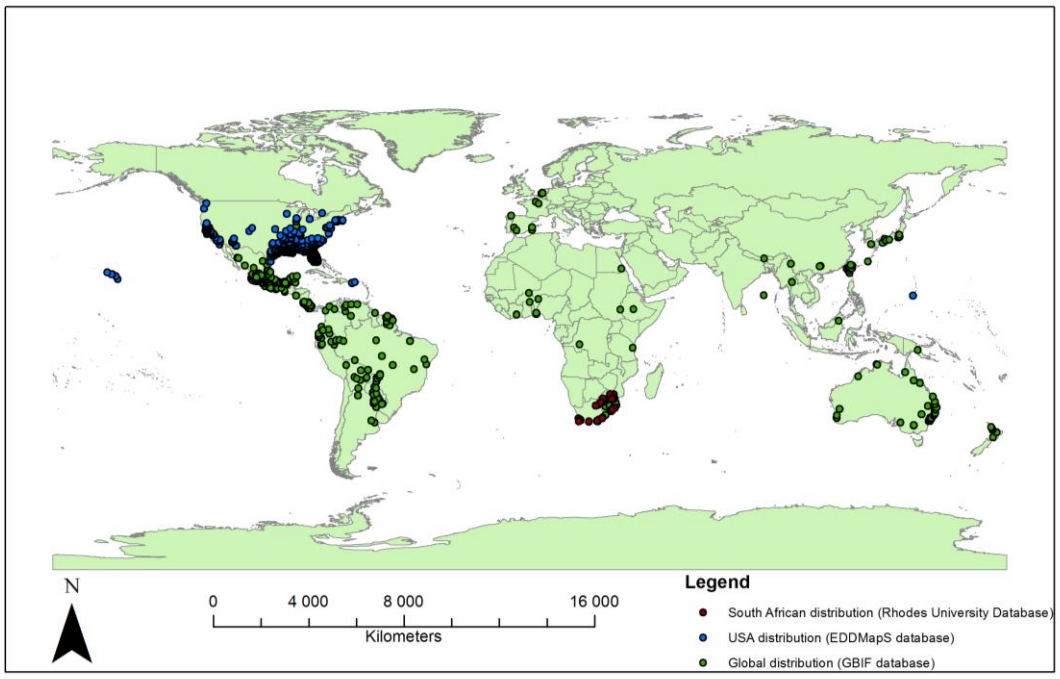


Figure 1.1: Global distribution of water hyacinth (data compiled from GBIF, EDDMapS, and Rhodes, Department of Zoology and Entomology from Smit, 2013)

Water hyacinth has severe ecological and socio-economic impacts. Because of its dense, interlocking mats, which have been recorded to extend up to 75 km across a single water surface (Ruiz Téllez *et al.*, 2008), water hyacinth is known to displace native flora, prevent light penetration, and deplete and obstruct oxygen supplies causing significant changes in the invertebrate composition and primary productivity (Timmer and Weldon, 1966; Toft *et al.*, 2003; van der Heide *et al.*,

2006; Jones, 2009; Villamagna and Murphy, 2010). Water hyacinth mats may also cause increased siltation, impede navigation, and block drainage in water bodies, contributing to flooding and even infrastructure damage (Timmer and Weldon, 1966; Mailu, 2001; Osumo, 2001). Water hyacinth infestations further impact nearby human population by limiting water access and quality (Mailu, 2001; De Groote *et al.,* 2003) and increasing the potential health risks associated with infested water bodies (Garcia and Huffaker, 1979; Mailu, 2001). Water hyacinth remains South Africa's worst aquatic weed(Coetzee *et al.,* 2011), affecting both ecological and human communities; hence, investigations to improve its control and management are urgently needed.

Growth of water hyacinth

Water hyacinth reproduces both vegetatively and sexually. Although water hyacinth spreads predominantly through vegetative growth, it can produce seed banks of up to 4228 seeds/m^2 (Albano Pérez *et al.,* 2011*a*). In order to germinate, water hyacinth seeds require warm, shallow waters and high light intensities (Center and Spencer, 1981). It germinates very quickly (within 3 days, Albano Pérez *et al.,* 2011*a*) but nutrients such as Nitrogen, Phosphorus and Boron may also influence germination times (Albano Pérez *et al.,* 2011*b*). Once seeds have germinated, submerged seedlings root in the substrate and begin to develop leaves, which contain aerenchyma tissue. When the plant is sufficiently buoyant, it breaks off the rootstock and floats on the open body of water (Penfound and Earle, 1948; Center and Spencer, 1981). Leaves continue to form from the apical bud, while fibrous adventitious roots develop on the stem at the base of leaves. Because of the rosette formation of water hyacinth leaves, leaves are often numbered from youngest (nearest the middle of the plant) to oldest (towards the edge of the plant) leaf.

Ramets, which are vegetatively produced plants, are formed on the axillary buds, forming water hyacinth mats. Although leaf production occurs at a regular rate, the leaf form varies with mat density (Figure 1.2). Bulbous leaf forms are found toward the edge of a mat while elongate leaf forms are found toward the middle

part of the mat when crowding occurs (Penfound and Earle, 1948; Center and Spencer, 1981). Mat density and population growth are affected by multiple factors.

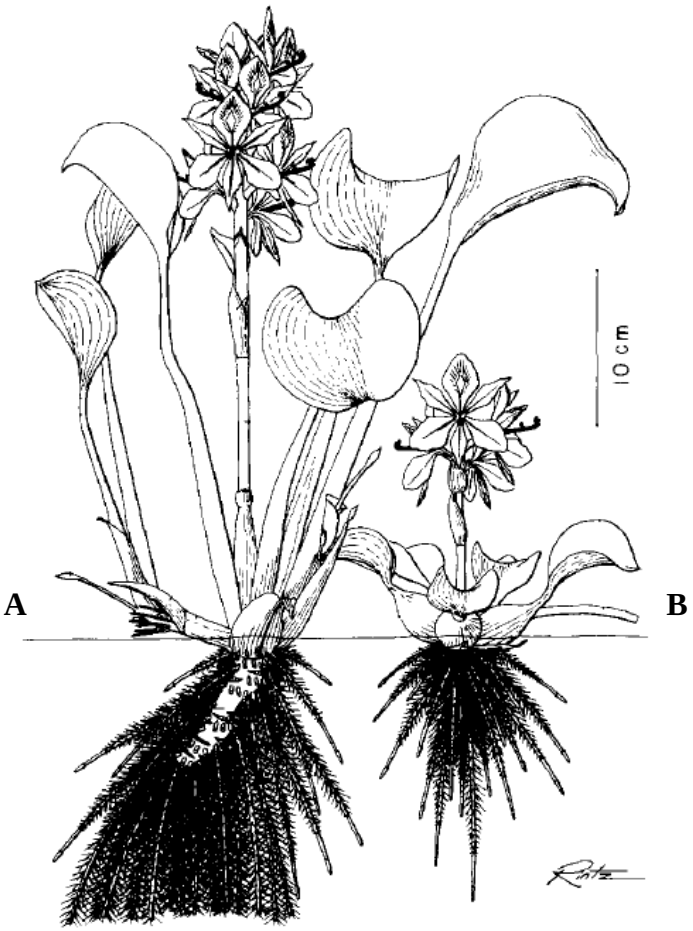


Figure 1.2: A generalized sketch of water hyacinth plants showing the growth form which occurs in A. dense mats as compared to the growth form that occurs in B. more open situations (After Center and Spencer, 1981).

Factors affecting growth of water hyacinth

As with the growth of any organism, many factors affect the growth of water hyacinth. Temperature, nutrients, plant density, water movement, frost, carbon dioxide concentration, humidity, light, pH, and salinity all affect the growth of water hyacinth, but to different extents (Haller and Sutton, 1973; Haller *et al.*, 1974; Reddy and DeBusk, 1984; Idso *et al.*, 1987; Sato, 1988; Carr *et al.*, 1997; Wilson *et al.*, 2005; Byrne *et al.*, 2010). The growth rate of water hyacinth is also

significantly affected by the plant biomass available per unit area. As water hyacinth densities increase, growth rates decrease (Reddy and DeBusk, 1984; Sato, 1988). As such, the growth of water hyacinth is density-dependent. The density and growth of water hyacinth mats, however, is also constrained by the size of the water body, water movement (Wilson *et al.,* 2005; Byrne *et al.,* 2010), as well as water salinity and pH, all of which can constrain growth or be lethal to the plant (Haller and Sutton, 1973; Haller *et al.,* 1974).

Frost directly affects plant growth by causing major leaf mortality (Wilson *et al.,* 2005; Byrne *et al.,* 2010). However, relationships between water hyacinth growth and frost are not as well understood as the effects of temperature and nutrients. Temperature and nutrients are considered as the major determinants of water hyacinth growth (Lorber *et al.,* 1984; Wilson *et al.,* 2001; Wilson *et al.,* 2005; Byrne *et al.,* 2010).

Temperature – Virtually all physiological processes are affected by temperature, accelerating as temperatures increase and declining as they decrease (Sato, 1988). The growth of water hyacinth is one such process, driven mainly by water temperature (Sato, 1988; Gutiérrez *et al.,* 2001; van der Heide *et al.,* 2006). Growth rates differ significantly, depending on the water temperature of the system (Reddy and DeBusk, 1984; Sato, 1988). Growth is a combination of multiple physiological processes that can function at a maximum rate, which occurs at an optimal temperature, or range of temperatures (Carr *et al.,* 1997). As such, growth of water hyacinth has both upper and lower water temperature limits (38-40°C and 8-10°C respectively) (Penfound and Earle, 1948; Urbanc-Bercic and Bagerscik, 1989; Wilson *et al.,* 2005; van der Heide *et al.,* 2006; Byrne *et al.,* 2010), outside of which water hyacinth growth and biomass densities will rapidly decline (Carr *et al.,* 1997; Wilson *et al.,* 2005).

Nutrients – Nutrients, particularly Nitrogen and Phosphorus, have a distinct effect on water hyacinth growth (Lorber *et al.,* 1984; Reddy *et al.,* 1989; Reddy *et al.,* 1990; Heard and Winterton, 2000; Wilson *et al.,* 2005; Coetzee *et al.,* 2007*a*).

Increasing water Nitrogen levels increases growth rate of water hyacinth (Reddy *et al.,* 1989), while growth rate and nutrient uptake is also greatly increased by increasing Phosphorus concentrations (Reddy *et al.,* 1990). Many experiments have considered the growth of water hyacinth affected by nutrients, but rarely have water temperatures been incorporated at the same time (Reddy and Tucker, 1983; Reddy and DeBusk, 1984; Reddy *et al.,* 1989; Reddy and D'Angelo, 1990; Reddy *et al.,* 1990; Ripley *et al.,* 2006).

There are many eutrophic water bodies in South Africa (caused by increasing pollution from nearby industries and increased urbanisation) (Oberholster and Ashton, 2008), promoting the growth of water hyacinth. Nutrients in aquatic systems are highly variable, depending on location and season (Perona *et al.,* 1999). This variability is likely to impact both the plant and its interactions with herbivores in the system, through its effects on plant growth rate (Reddy *et al.,* 1989; Reddy *et al.,* 1990; Wilson *et al.,* 2005) and plant nutrient content, which drives herbivory and herbivore populations (Heard and Winterton, 2000; Coetzee *et al.,* 2007*a*; Center and Dray, 2010*a*).

Herbivory – Herbivory by agents, such as *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache, removes biomass from water hyacinth. Adult weevils feed on the external plant surfaces decreasing the available photosynthetic area and photosynthetic productivity (Spencer and Ksander, 2004; Venter *et al.,* 2013) while larvae tunnel inside the petiole (DeLoach and Cordo, 1976; Forno, 1981; Jianqing *et al.,* 2002) reducing plant buoyancy. With the reduction in buoyancy and photosynthetic capabilities, water hyacinth plants cannot grow as successfully, causing declines in the population of the plant. Although herbivory can result in massive reductions of the plant population such as seen in Lake Victoria (De Groote *et al.,* 2003; Wilson *et al.,* 2007), Soti and Volin (2010) show that (simulated) herbivory needs to remove more than 10% and possibly up to 80% of the lamina before causing significant decreases in the relative growth rate of the plant. Furthermore, herbivory is frequently related to host plant quality, increasing with increasing nutrients

(Moran, 2004; Center and Dray, 2010*a*; Franceschini *et al.,* 2010). However, the host plant quality changes quickly because of fluctuations in nutrients in the environment (Hill, 2014). As a result, biocontrol agents are constantly adjusting their reproductive capacities to variable host quality (Center and Dray, 2010*a*). This has been cited as one of the reasons for variable control of aquatic invasive plants such as water hyacinth (Center *et al.,* 1999*b*; Hill and Olckers, 2001; Center and Dray, 2010*a*).

Control of water hyacinth

Control of water hyacinth populations is a major concern for water managers and has been the primary focus of water hyacinth research in South Africa for many years. Many of the current water hyacinth control methods have limitations, and often an integrated approach combining biological and chemical control is used to achieve management goals (Charudattan, 1986; Jadhav *et al.,* 2008). Although chemical control provides immediate results, it is often unsatisfactory in the long term. Water hyacinth spreads predominantly through vegetative growth, and can produce large seed banks (Albano Pérez *et al.,* 2011*a*); however, applications of herbicide can bring about rapid reductions in water hyacinth mats. This not only results in large volumes of decomposing detritus, returning nutrients and other elements to the water bodies (Lugo *et al.,* 1998; Gupta *et al.,* 1996; Reddy and DeBusk, 1991), but also allows light to filter through to the sediment. Increased light stimulates water hyacinth seed germination (Center and Spencer, 1981), resulting in a resurgence of the water hyacinth population. Aside from population resurgence, chemical control also has ecological impacts of its own. Lugo *et al.,* (1998) noted that chemical control had a direct toxic effect on phytoplankton communities. Changes in phytoplankton growth and density result in a reduction in zooplankton, which is likely to have knock-on effects throughout the food chain. They also noted that dissolved oxygen levels were depleted as a result of the decomposing water hyacinth detritus in the water body. Consequently, chemical control is not ideal in many aquatic ecosystems, especially in Africa where many human populations depend directly on affected water bodies.

Biological control is considered to be a cost effective, long-term approach to water hyacinth control (van Wyk and van Wilgen, 2002; Hoelmer and Kirk, 2005; Law, 2007; van Wilgen and De Lange, 2011), using natural enemies to reduce infestations. Biocontrol agents are subject to rigorous host specificity testing and regulations (Ruesink *et al.,* 1995; Moran *et al.,* 2005), and have fewer ecological impacts than other control methods (Villamagna and Murphy, 2010). Although eradication of the problem weed (such as water hyacinth) is seldom possible, biocontrol systems can keep the target weed populations at manageable levels, because of their self-regulating nature. Stochastic events, however, are extremely important in biological control of water hyacinth. This is because events such as flooding and frost remove water hyacinth from the system, and as a result remove biocontrol agents which they harbour (Wilson *et al.*, 2001; Byrne *et al.,* 2010). Resurgence of water hyacinth populations, from seed banks in the sediment or any surviving rootstocks, will occur as soon as favourable conditions return. Control agent populations, however, will have generally been eliminated and take much longer to recover, allowing water hyacinth populations to spread unimpeded (Byrne *et al.,* 2010). As a result, in many countries such as South Africa, water hyacinth infestations remain a problem even with a suite of biocontrol agents released against the plant (Jones, 2001; Coetzee *et al.,* 2011; Coetzee and Hill, 2012).

1.3.2 *Neochetina* water hyacinth weevils

Although many biological control agents have been released against water hyacinth, to date *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache are considered the most effective (DeLoach and Cordo 1976; Center and Van, 1989; Center *et al.,* 1999*b*; Ajuonu *et al.,* 2009; Center *et al.,* 2014). These weevils, as adults, feed on the leaf lamina, petioles, and stem bases (Spencer and Ksander, 2004) and lay their eggs in the leaves and petioles (DeLoach and Cordo, 1976; Shih *et al.,* 1994). This allows larvae to tunnel to the crown of the water hyacinth plant (DeLoach and Cordo, 1976; Forno, 1981; Jianqing *et al.,* 2002) destroying petiole tissue and decreasing plant buoyancy. As such, larvae are considered the most damaging life stage of the weevil biocontrol

agent's lifecycle (Wilson *et al.*, 2001; Wilson, 2002; Wilson *et al.*, 2005; Ripley *et al.*, 2008). Mature larvae attach to the living rootstock of the plant underwater where they pupate (DeLoach and Cordo, 1976). Once the adults emerge, they begin feeding on the lamina, creating feeding scars, which reduce photosynthetic area of water hyacinth (Franceschini *et al.*, 2010; Soti and Volin, 2010) and introduce pathogenic agents which contribute to reductions in photosynthetic productivity (Ripley *et al.*, 2008; Venter *et al.*, 2013). Although *Neochetina* weevils can have devastating effects on water hyacinth populations, reducing some water hyacinth populations by up to 95% (Jayanth, 1988), in many areas of South Africa control results have been inadequate, resulting in the continuation of active management, usually with herbicides, at many locations (Byrne *et al.*, 2010).

Variability in the effect of biological control in South Africa has also been attributed to nutrient rich waters and sub-optimum water temperatures for *Neochetina* control agents, increasing water hyacinth populations beyond that which biocontrol could possibly have an effect (Hill and Olckers, 2001). Water hyacinth plants also tend to absorb heavy metals, such as Cadmium, Lead and Mercury (Muramoto and Oki, 1983) that affect the fecundity of biocontrol agents (Jamil and Hussain, 1993; Newete *et al.*, 2014). These factors, as well as the hydrology of small water bodies, flooding events, climate incompatibility and the extensive use of herbicides in South Africa have also been attributed as the cause of variability in success of biological control (Cilliers, 1991; Coetzee *et al.*, 2007*b*).

Factors affecting Neochetina weevil growth

The growth and reproduction of biological control agents (including Neochetina weevils) is influenced by several factors, such as weather, disease, predators, and plant quality, which have knock-on effects on the agents' establishment and performance (Newman *et al.*, 1998). Weevil agents of water hyacinth are adversely affected by several environmental factors. Stochastic events such as drought, flooding, frost events, herbicide application, as well as other climatic

conditions and host plant quality have been shown to influence weevil populations drastically (Wilson *et al.,* 2001, Center and Dray, 2010*a*, *b*).

Both drought and flooding events reduce weevil populations by removing weevils from the system (Wilson *et al.,* 2001). Drought causes desiccation of water hyacinth, removing food sources for *Neochetina* weevils. Although weevils may survive for a period under drought conditions, muscle development is retarded (as a result of starvation) and adults are unable to migrate from desiccating plant populations (Jayanth and Visalakshy, 1990).

Frost events and application of foliar herbicides may also result in reduced weevil populations. Leaf dynamic processes, available leaf area and some portion of the petioles are severely and rapidly impacted and reduced by frost and herbicides. Herbicide applications not only remove adult feeding sites, but also affect the egg population. *Neochetina* eggs are normally found in the top portion of the petiole, below the lamina and are likely to be killed by severe frost events. First and second instar larvae populations occupying the upper petioles are also likely to be reduced with the reduction in viable petiole tissue. Reducing viable leaf and petiole quantity can have disproportional effects on weevil populations, especially with relatively large populations of young larvae (Wilson *et al.,* 2006; Byrne *et al.,* 2010).

Although floods, droughts, herbicide application and frost events affect weevil populations, these events are stochastic. Climatic conditions and plant host quality are considered the non-stochastic major factors affecting *Neochetina* weevil populations.

Climatic conditions

Climatic conditions, such as cold winters, have a large effect on weevil populations, by upsetting leaf dynamics causing disruptions in egg and larvae populations, as well as reducing the rate of development (Julien, 2001; Wilson *et al.,* 2001). Insect development depends on the temperatures to which the

immature stages are exposed (Campbell *et al.,* 1974). As such, exposure to temperatures below ca. 15°C often slows or stops *Neochetina* development (Julien, 2001), slowing population growth. Temperature further affects the feeding and fecundity of *Neochetina* weevils (Njoka *et al.,* 2006; Byrne *et al.,* 2010). High temperatures (above the 30°C optimum) may cause egg production to decrease as well as reduce adult survival (Julien, 2001; Oke, 2008), while low temperatures may cause follicle re-absorption, reducing reproductive ability (Byrne *et al.,* 2010). Reductions in fecundity and weevil survival often have detrimental effects on the population.

Host plant quality

Host plant quality and nutrient availability are arguably the most important drivers of *Neochetina* weevil populations, with numerous accounts of increasing fecundity of weevils with increasing plant quality (Center and Dray, 1992; Center, 1994; Center *et al.,* 1999*a*; Heard and Winterton, 2000; Center and Dray, 2010*a*). Low nutrient availability and poor host quality have been linked to female weevils switching from reproductive to dispersive modes (Center and Durden, 1986), which would result in local population declines. However, adequate nutrition is required for this transition to occur (Center and Dray, 2010*b*). Although plant quality may not directly affect survival of *Neochetina* weevils, at higher nutrient concentrations development of larvae is faster, allowing for rapid increases in weevil populations (Julien, 2001; Wilson *et al.,* 2006).

1.3.3 Modelling biological control systems

Although important advances have been made in various aspects of biological control (Barratt *et al.* 2010), biological control programmes have been criticised for their historical trial-and-error approach to agent selection (Mills and Kean, 2010) and lack of rigorous evaluation of agent impacts. However, different theoretical models, such as population models, systems models, mechanistic models and empirical models, can play a significant role in the evaluation of biological control of weeds. These models serve as useful tools that provide frameworks for designing appropriate experiments, predicting agent impacts,

exploring relationships and interactions in the system and determining guidelines for practical weed management (Kriticos, 2003; Sims *et al.*, 2006; Holst *et al.*, 2007; Sheppard *et al.*, 2003; Morin *et al.*, 2009; Mills and Kean, 2010). Models can be used to demonstrate that weed populations have declined because of biocontrol agents rather than other external factors (Sims *et al.*, 2006). Furthermore, they are heuristic tools which provide insight into understanding the mechanisms that underlie the success and failure of biocontrol programmes (Kriticos, 2003; Sheppard *et al.*, 2003; Holst *et al.*, 2007), as well as the ecosystem impacts of both invasive plants and the methods used to control them (Ewel *et al.*, 1975). Understanding the success, failure and impacts of alien infestations and invasive control is fundamental in determining effective solutions to this intractable problem.

Climate is often one of the most limiting factors, affecting many biological control agent populations (McEvoy and Coombs, 2000; Zalucki and van Klinken, 2006), particularly in water hyacinth control in South Africa (Hill and Cilliers, 1999; Byrne *et al.*, 2010; Coetzee *et al.*, 2011). However, these conditions are not stable and are expected to change in the future. In South Africa, air temperatures are expected to rise between 3 - 4°C and precipitation is expected to decrease by up to 20% in some areas of the country, (UK Met Office, 2012). As a result, understanding the influences of climate on population dynamics and control efficacy is important in making biocontrol as successful and cost effective as possible.

Climate matching models, such as CLIMEX®, are often used to aid biocontrol programmes by helping identify new areas for exploration for new agents (Senaratne *et al.*, 2006), simulating agent's potential distribution and their subsequent population dynamics (Coetzee *et al.*, 2007*b*; Lawson *et al.*, 2008). However, simple climate matching can be misleading, and produce results that do not necessarily reflect reality (van Klinken *et al.*, 2003). As a result, population interactions with climate and other environmental factors should be modelled in more detail to ensure that accurate predictions can be made to inform biological

control and management decisions. Including population dynamics of both the agent and the target and the influence of climate on both will result in robust models that can be used to predict agent and target populations under changing climate conditions.

There are a number of types of models used in biological control, but the most frequently used are population models. Population models are considered to offer an excellent approach to weed ecology as they include the life cycles and population dynamics of the species involved, and can have added levels of complexity (e.g. germination response). Furthermore, these models can also include spatial heterogeneity or distribution (van Groenendael, 1988), producing models which can predict the size of populations as well as the geographical extent.

Types of population models

There are a number of different types of population models, namely population growth models (logistic and exponential), competition models, and predator-prey/consumer-resource models (Otto and Day, 2007). Exponential and logistic growth models are the simplest models that describe changes in population sizes and ignore interactions with other species. The difference between these two simplistic models lies in their assumptions regarding the resources available to a population. Logistic models assume limited resources for each individual in a population (density dependence) reaching a system carrying capacity (maximum population size), while exponential models assume that every individual will have access to the same resources, regardless of the population size (Figure 1.3; Otto and Day, 2007).

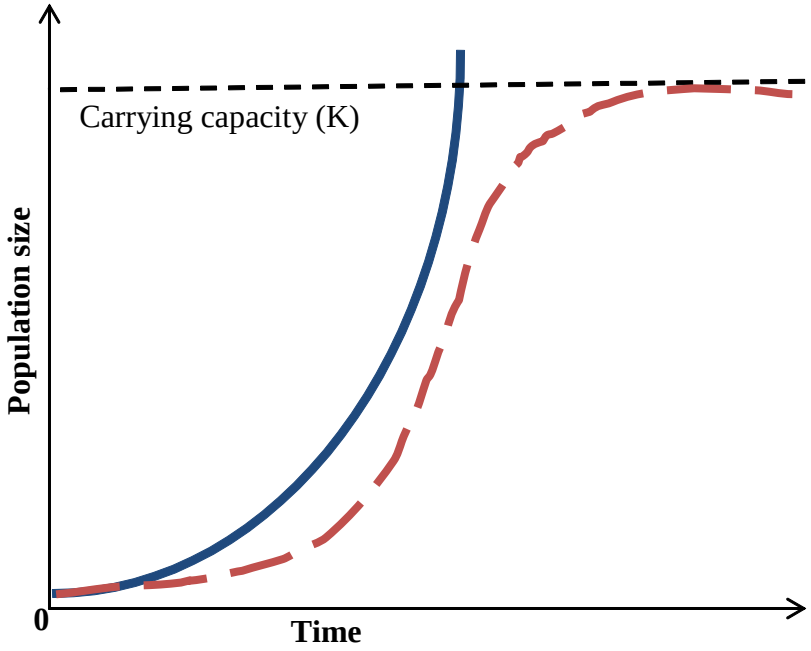


Figure 1.3: Logistic (dashed red) versus exponential (solid blue) growth population models

As such, the logistic model is often used to approximate plant populations. Multiple species of plants often occur within communities, and as a result, models of competition (for resources) are used. Competition occurs within (intraspecific) and between (interspecific) species. Although interspecific competition occurs in most indigenous communities, infestations of alien plants, particularly water hyacinth, often have no competitors. The logistic model is thus used in many invasive aquatic population models. With the application of biological control, however, weed populations cannot be predicted using logistic models alone, as these models tend to ignore the influence of herbivory. Lotka-Volterra predator-prey models or consumer-resource models are incorporated to include herbivory effects. Both of these models are used when the resources of a population are affected by the growth of that population (Otto and Day, 2007). Predator-prey models are used in simulating biocontrol systems, as the biocontrol agent can be considered as the "predator" while the target weed is the "prey".

Population models of **Eichhornia crassipes**

Although several models of water hyacinth growth have been developed, each has been constructed to achieve a different goal. These goals range from the production of biomass for methane, to the control of water hyacinth as an invasive aquatic plant (Lorber *et al.,* 1984; Akbay *et al.,* 1991; Wilson *et al.,* 2001). Although mostly successful in their particular aims, these models cannot be used effectively in management and control of water hyacinth as an aquatic pest. This is often because several factors affecting water hyacinth and weevil populations and growth are neglected. Below, various models of water hyacinth populations are discussed with respect to their application in aquatic weed control.

Plant growth models

Variations of logistic growth models have proven to be useful in understanding water hyacinth growth (Wilson *et al.,* 2005) and for describing the extent of the weed invasion. However, these models may have limited application in aquatic weed control. Lorber *et al.,* (1984) created a model to evaluate the potential of large-scale water hyacinth biomass production for conversion to methane gas. Lorber *et al.,* (1984) simulated the potential maximum yield of water hyacinth and used it to determine an optimal harvesting strategy. Maximum sustainable yields provide some insight into the quantity of biomass that has to be removed before a population might decrease in size. In this case, with a starting density of 1 kg/m^2, the maximum sustainable yield was 63 tons/ha/year, suggesting that biomass removed by biocontrol agents needs to exceed this to reduce water hyacinth populations (Lorber *et al.,* 1984).

While Lorber *et al.,* (1984) showed their model to simulate field data particularly well, matching predicted versus observed biomass as close as 1%, the model parameters were extensive and included solar radiation, nutrients, maintenance respiration, and plant density. The model also required sub-models of Nitrogen and Phosphorus cycling in the plant as well as in the environment, making it more complicated and difficult to obtain the required inputs. This model was also specific to conditions in Florida, USA and did not include biological control.

Although excluding the effects of biocontrol prevents the model from being used effectively in water hyacinth control and management, particularly in areas under biological control, it can be used to determine optimal harvesting strategies in areas where biological control is not feasible and mechanical control predominates, such as the Northern states of the USA (e.g. New England) (US EPA, 2008).

Wilson *et al.*, (2005) developed a similar logistic growth model for water hyacinth. Likewise, it did not include herbivory, or biomass removal through various control methods. However, these authors did focus on temperature and water nutrient concentrations and their effects on water hyacinth growth rate, using mathematical modelling. Their model drew from a wide range of literature to estimate model parameters, and accurately described small-scale experiments. Nevertheless, its application to controlling invasive weeds is restricted. Describing small-scale experiments accurately does little to extend knowledge of growth of water hyacinth in its invaded areas where it is subject to fluctuating nutrient and temperature conditions, and in many cases, has the added stress of numerous control methods. Including the control methods as important influences on growth is vital in producing a tool that is useful in the global context of water hyacinth invasion. Wilson *et al.*, (2005) understood the importance of including biological control in their water hyacinth growth model. They state that "in a future paper [they] model the effects of temperature and nutrients on the interaction between water hyacinth and *Neochetina* spp., and so on the level of control achieved by these biological control agents", but to date this model has not been published.

Water hyacinth growth models provide insight into the problem of water hyacinth invasion across the globe but are limited by neglecting to include important factors, particularly herbivory. Removing plant biomass from a weed/agent system is essential, particularly in density-dependent systems, such as those described by logistic growth models. Creating a model of water hyacinth growth that incorporates the effects of variable environmental conditions, such as

temperature, as well as the impact of herbivory and other methods of biomass removal is vital to developing a tool that can simulate infesting populations effectively and help guide control and management decisions.

Weed control models

Chemical and mechanical control- Several models of water hyacinth have been created with some regard to water hyacinth management and control. To date, however, these models have not been developed sufficiently to be used to predict water hyacinth infestations to inform management and control decisions of water hyacinth infestations successfully. This may be due to the models not being tailored to the needs and background of water managers, particularly in South Africa.

The earliest of the chemical and mechanical control models was produced by Ewel *et al.,* (1975). Similar to Mitsch (1976), Ewel *et al.,* (1975) constructed a simple ecosystem model using Odum energy flows to demonstrate the usefulness of models in evaluating control strategies of aquatic weeds. Using water hyacinth as a case study, they demonstrated how chemical, and to some extent mechanical, control would influence water hyacinth populations and the ecosystems which they affect. This model predicted that reducing the rate of nutrient inputs within the system would decrease water hyacinth populations in the long term, while using an herbicide "partial-kill spray" may not have a long-term effect on the weed's populations. Although as an ecosystem model their model is simplistic, it still includes a substantial number of factors (oxygen, effective solar radiation, external N and P, dissolved N and P, algae and other phytoplankton, bottom rooted plants, water hyacinth, and detritus). However, many of these inputs are difficult to simulate and predict in complex water systems. This model also assumes a closed pond system limiting its application in river infestations.

Modelling mechanical harvesting of water hyacinth populations was further explored by Gutiérrez *et al.,* (2001). The aim of their project was to develop a tool that could describe the water hyacinth population and monitor the effect of

biomass harvesting. The model, however, neglects plant death, disease and herbivory through biological control, the last of which has become one of the key control methods of water hyacinth. It appears to have limited applicability in integrated control systems and has not included the direct influence of temperature and/or nutrients, which are by far the most important factors affecting growth in water hyacinth and most other aquatic plants (Lorber *et al.*, 1984; Wilson *et al.*, 2001; Wilson *et al.*, 2005; Byrne *et al.*, 2010).

Biological control models- The first authors to take cognisance of biological control and their potential impacts on their model were Forno and Bourne (1978). Because standing crop (the dry weight of leaf material per unit area) is considered an important factor in seasonal variation of water hyacinth growth, and subsequently biocontrol evaluation, Forno and Bourne (1978) developed an approach to estimate the standing crop of the plant. This produced estimates within 10% of the actual mean value of a standing crop. While they were aware that biocontrol by *Neochetina eichhorniae* affected the sites under consideration, the authors did not specifically include the weevil in the model. No measure of weevil population density or effect was included in determining the status of water hyacinth standing crops. This model was also restricted to plants of a particular height range and leaf frequency distribution. If any changes in the agent populations occurred, resulting in changing herbivory patterns, plant height and leaf distribution, the model would no longer be applicable. As such, this approach is unlikely to be used in modelling and controlling water hyacinth biocontrol systems.

Wilson *et al.*, (2001) set out to construct a model that could be used as a predictive tool of water hyacinth control by *Neochetina eichhorniae*. This model used Lotka-Volterra equations to describe the relationship between plant and herbivore, as these equations had been used successfully in modelling the biocontrol of another aquatic weed (*Salvinia molesta*) (Room, 1990). Unfortunately, Wilson *et al.*, (2001) did not manage to produce a plausible model for predicting water hyacinth control because the model (Figure 1.4 A) diverged

extensively from field observations (Figure 1.5), by predicting eradication and "extremely low densities, which would effectively result in extinction" of water hyacinth. In addition, biologically, it is extremely unlikely that an herbivore would cause the extinction of its only host.

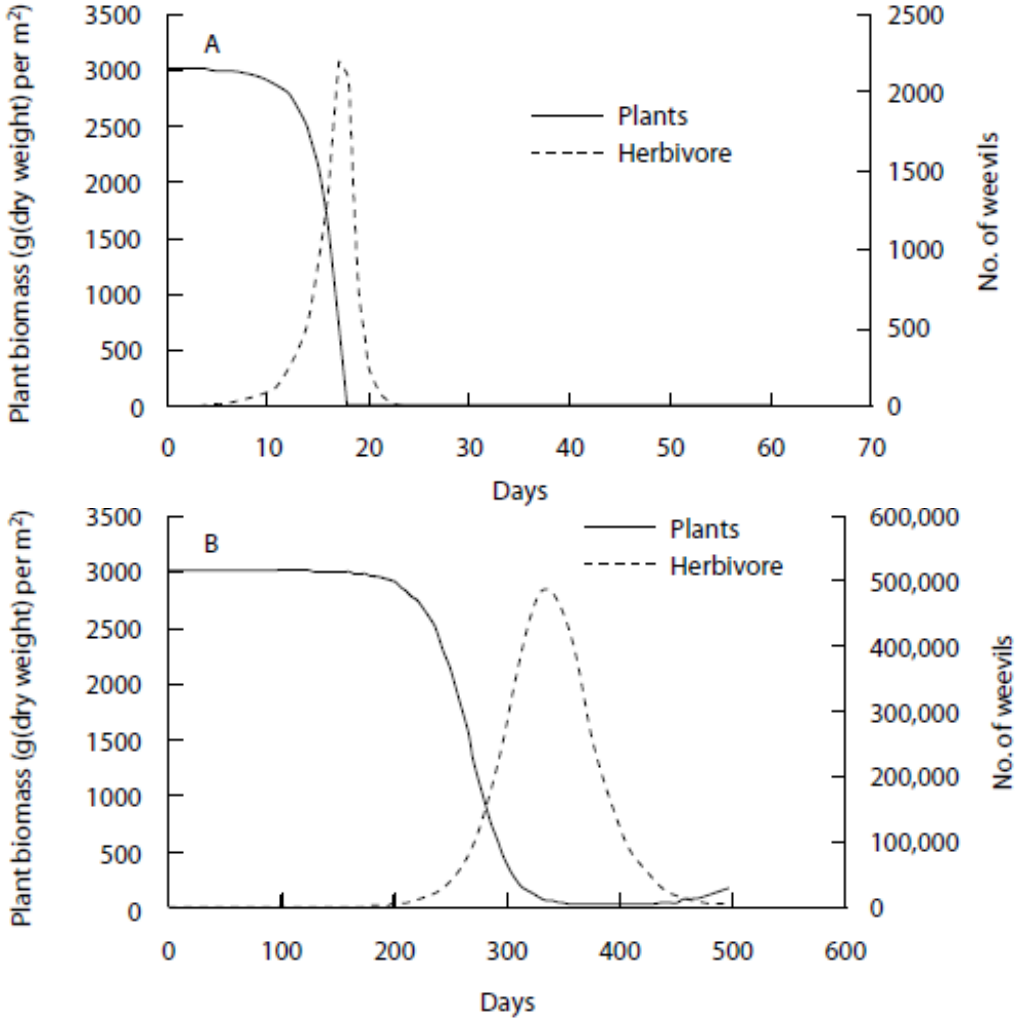


Figure 1.4: Wilson *et al.*, (2001) models of water hyacinth and weevil populations A. assumes all weevil stages have the same effect on the plant and B. includes a time delay to mimic larval damage to water hyacinth populations.

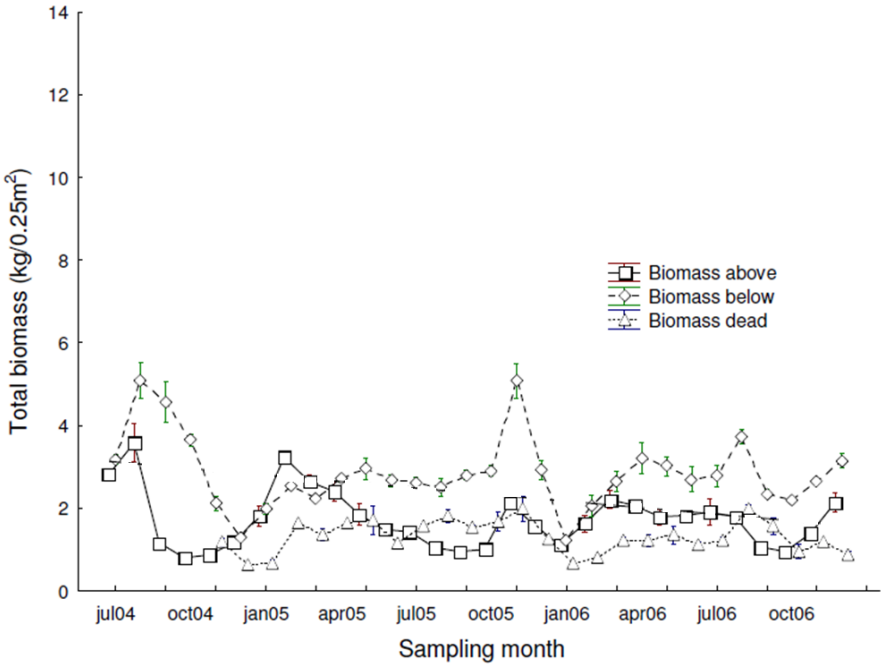


Figure 1.5: Monthly water hyacinth biomass at Delta Park, South Africa (After Byrne *et al.*, 2010). Compare the weed population over time with that in Figure 1.4 and note that the water hyacinth population does not go extinct as in Figure 1.4.

It is likely that the model predicted extinction (Figure 1.4 A) because other important environmental factors (such as temperature) were not incorporated into the model. Although the authors discuss the limitations of their model, as well as control by weevils, to date the model has not been developed further.

The most coherent water hyacinth biocontrol model to date is that of Akbay *et al.*, (1991). They developed a computer-modelling programme called INSECT with the aim of using it as a predictive tool for determining and evaluating the impact of biological control (by *Neochetina* weevils) on water hyacinth populations. The INSECT model incorporated models of plant growth similar to those produced by Lorber *et al.*, (1984) and a population module for both *Neochetina eichhorniae* and *N. bruchi* and assumed that water nutrients were not limiting. This model proved to be mostly effective by simulating populations within 95% confidence intervals of field-collected data for the growing seasons. However, the INSECT model only simulated a single year and diverged from field data in the early and

late stages of the year. Although the model was to be developed further, the project ended preventing any major improvements (Howell and Stewart, 1988).

1.3.4 The way forward

Water hyacinth remains one of the most invasive aquatic weeds internationally, and is still a major problem in South Africa. Understanding the dynamics of this pest is vital in determining management and control strategies. Although models can be used effectively to understand and manage invasive species populations, to date no successful model has been developed to predict management implications of water hyacinth populations. The development of a model that can predict potential water hyacinth and weevil populations will be instrumental in evaluating the potential threat of the weed and what control strategies are most suited to a particular site.

In this dissertation, a simple model of water hyacinth biocontrol will be developed. This model will include temperature, one of the most important determining factors of both water hyacinth and weevil populations. Although temperature is often neglected in modelling, temperature data are easily obtained by water managers. This model will also be stage-structured, incorporating the differential herbivory effects of *Neochetina* weevil life stages, because the larval stage is considered to be more damaging than is the adult (Bashir *et al.,* 1984).

The model in this project will be used to simulate water hyacinth and weevil populations, and is aimed to be accessible to researchers and water managers alike, if not as a functioning tool then as decision framework. It forms part of a larger project that aims to develop a temperature and nutrient driven model, capable of simulating water hyacinth populations across the globe as well as assessing the risk to water hyacinth biological control from climate change.

1.3.5 Structure of the dissertation

Chapter One consists of a general introduction, including a brief review of the literature on water hyacinth and its growth, *Neochetina* weevils and the factors affecting their growth, and modelling populations, particularly in biological control systems.

Chapter Two briefly reviews the literature on insect development, insect survival, herbivory and temperature. It describes the experiments carried out to determine the effect of temperature on *Neochetina eichhorniae* egg development and survival, the effect of larval feeding at 25°C and the effect of temperature and nutrients on adult weevil feeding (Objective 2). These experimental results are then used in the construction of the water hyacinth biological control model in Chapter Three.

In Chapter Three, the types and uses of models in biological control as well as model population dynamics and parameterisation are reviewed. The methods for constructing a stage-structured systems model of water hyacinth biological control are shown (Objective 1, 2, 3). Models were created in stages, with each stage being run for a period of two years simulating two South African sites, Mbozambo Swamp (29°21’S, 31°18’E), and Delta Park (26°07’S, 28°00’E), which are representative of the warmest and coldest sites sampled in Byrne *et al.* (2010).

A short summary of the literature on model validation is included in Chapter Four. The chapter continues to explain how final stage models were validated against independent, seasonal observed water hyacinth and weevil population data for the two sites (Objective 4).

Chapter Five provides a general discussion about how modelling has shown the importance of temperature in water hyacinth/weevil biological control systems. It also discusses the inherent flaws and benefits of the model created in this study and draws on these results to suggest improvements for future models.

2 CHAPTER TWO – TEMPERATURE-DEPENDENT DEVELOPMENT AND FEEDING OF *NEOCHETINA EICHHORNIAE*

2.1 Introduction

2.1.1 Temperature and insect development

The process of development, in any organism, involves intense metabolic reactions, the rates of which are limited by the temperature-dependence of the slowest step (Ratte, 1985). Insects generally have very low body weights, resulting in low heat capacities and thermal inertia (Jankowsky, 1973). They are therefore unable to maintain constant body temperatures, meaning that their growth and development is largely dependent on external conditions (Higley and Haskell, 2002). The relationship between insects and temperature has been considered by scientists for centuries (Réaumur, 1735; Higley and Haskell, 2002) and has been concluded as the principal determinant of behaviour and physiology of insects during all developmental stages (Liu *et al.*, 1995).

The rate of growth and development of insects generally increases with increasing temperature, but only within an optimal temperature range (Sharpe and DeMichele, 1977; Taylor, 1981; Hartley and Lester, 2003). There are both upper and lower developmental temperature thresholds, outside of which development is drastically slowed, if not stopped entirely. Describing the process of development and determining developmental thresholds can be done experimentally, normally by measuring the time taken to complete a developmental event at a given temperature (Wagner *et al.*, 1984; Laudien, 1973). Such experiments not only describe developmental processes but also generally result in estimates of upper and lower lethal temperatures (Mitchell *et al.*, 1993), developmental rates and survival proportions (Rueda *et al.*, 1990), developmental thresholds (McAvoy and Kok, 1999) and species-specific thermal constants (Damos and Savopoulou-Soultani, 2012).

Being able to relate developmental rates to temperature has resulted in numerous attempts at modelling the relationship, which has been used extensively in estimating insect phenology, particularly for economically important species (Wagner *et al.*, 1984; Aurambout *et al.*, 2009; King, 2011; Zuo *et al.*, 2011).

2.1.2 Line-fitting methods for the estimation of degree-day thresholds

For more than 250 years, temperature has been used to describe life-history events across insect species (Réaumur, 1735; Sharpe and DeMichele, 1977; Higley *et al.*, 1986, Rueda *et al.*, 1990; Hartley and Lester, 2003). Various methods of describing temperature growth relationships have been developed (Wagner *et al.*, 1984; Régnière *et al.*, 2012), most of which express insect development in terms of thermal units called degree-days (°D; Campbell *et al.*, 1974; Lactin *et al.*, 1995; Ikemoto and Takai, 2000). The linear intercept method was proposed by Campbell *et al*. (1974), who approximated the line as

$$y = a + bT$$

(Equation 1)

where y is the developmental rate (1/day) and T is the insect rearing temperature (°C). Although this method is relatively accurate and simple to use, it was questioned by Ikemoto and Takai (2000), who described three shortcomings of the method and concluded that it resulted in unreliable estimations of developmental thresholds (*t*), the temperature below which insect development is halted, and degree-day requirements (*K*), the developmental duration from egg to adult. Ikemoto and Takai (2000) subsequently suggested an alternative method, the reduced major axis regression method.

The reduced major axis regression method (Ikemoto and Takai, 2000) is represented by the straight line

$$DT = K + tD$$

(Equation 2)

where *D* is the developmental duration and *DT* is the product of this developmental time and the corresponding temperature (°C). It has therefore been used to determine relevant temperature-dependent variables of *t* and *K*, which are crucial in estimating temperature-dependent development and understanding insect population dynamics (Sutherst and Maywald, 1985), particularly in biocontrol and pest-prone systems (Liu *et al.*, 2002; Gillespie *et al.*, 2004; Goebel, 2006; Coetzee *et al.*, 2007*b*).

Wilson (2002) maintains that larvae are the most damaging life stage of the *Neochetina* weevil biocontrol agents of water hyacinth. Therefore, understanding the timing and potential density of larval populations is imperative when estimating the effect of biocontrol on water hyacinth infestations. In order to estimate larval populations, a firm understanding of how temperature affects the survival and development of *Neochetina* eggs is needed. Temperature-dependent egg experiments and the reduced major axis regression method will thus be used to determine the developmental threshold and degree-day requirements for hatching to occur, allowing larval populations to be estimated in a stage-structured population model of *Neochetina eichhorniae*.

2.1.3 Temperature and insect herbivory

As well as influencing insect development, temperature also affects insect oviposition, longevity (McAvoy and Kok, 1999), survival between life stages (Shima and Hirose, 2002), and feeding (Forno and Bourne, 1985). As temperatures increase the resting or basal metabolism increases, usually leading to increased activity and energy requirements (Wigglesworth, 1974). At higher temperatures, insects are generally more active, develop faster, require more energy and thus may consume food at a higher rate. Numerous investigations have been undertaken to determine how insect feeding rates change under different temperature conditions (DeLoach and Cordo, 1976; Ferro *et al.*, 1985; Lactin and Johnson, 1995; Chikwenhere, 2000). Understanding feeding rates is particularly important for biocontrol, which often relies on the feeding behaviour of agents to

help reduce the target organisms' population (Samways and Wilson, 1988; Stiling and Cornelissen, 2005).

Although water nutrients and subsequently plant quality influence the effectiveness of *Neochetina* weevils (Heard and Winterton, 2000; Moran, 2004; Center and Dray, 2010*a*; Franceschini *et al.*, 2010), the interacting effect of temperature with nutrients on weevil feeding has not been considered (DeLoach and Cordo, 1976; Chikwenhere, 2000; King, 2011). However, temperature/plant quality interactions have been considered in other insects (Stamp and Bowers, 1990; Lindroth *et al.*, 1997; Levesque *et al.*, 2002; Paritsis and Veblen, 2010) showing that plant quality can influence the effect of temperature on insect feeding and growth. Lindroth *et al.*, (1997) showed that consumption rates of the gypsy moth, *Lymantria dispar*, increased with temperature but decreased with higher dietary nitrogen levels, while Levesque *et al.*, (2002) showed that consumption rates of the caterpillar *Malacosoma disstria* increased with both increasing temperature and plant quality. Lee and Roh (2010) also show a significant interaction between temperature and diet of *Spodoptera exigua*. Different relationships between temperature and nutrients exist for different species, thus understanding how temperature and nutrients or plant quality interact to influence *Neochetina eichhorniae* feeding is particularly important in biological control systems.

Little effort has been made to quantify the effect of larval herbivory on water hyacinth biomass (Chikwenhere, 2000), despite larvae being considered as the most damaging life stage of the *Neochetina* weevils (DeLoach and Cordo, 1976, Bashir *et al.*, 1984). As such, a first attempt to quantify larval biomass removal at 25°C will be made here. Together with the effects of temperature on egg development, egg survival, and adult feeding, these relationships will be used in models to determine water hyacinth biological control (Chapter 3).

2.1.4 Objectives

The objectives of this chapter are as follows:

1) Determine hatching times and survival proportions for *Neochetina eichhorniae* eggs at different temperatures.
2) Calculate the developmental threshold (*t*) and thermal constant (*K*) for *N. eichhorniae* eggs.
3) Quantify biomass removal of water hyacinth by *N. eichhorniae* larvae at 25°C.
4) Determine feeding rates of *N. eichhorniae* adults at different temperature and nutrient levels.

2.2 Methods

2.2.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs

Neochetina eichhorniae weevils were placed into 1.8L sealed plastic tubs with three to six water hyacinth leaves and allowed to oviposit at 25°C overnight. Before dissecting, leaves and dissecting utensils were surface-sterilized, using household bleach, diluted 2:1 (Jik, 3.5% sodium hypochlorite). Eggs were then dissected out from the leaves and placed onto moistened filter paper in Petri dishes, to prevent desiccation. The filter paper and deionised (DI) water had been heat sterilised. Petri dishes were placed into 1.8L sealed plastic tubs lined with damp paper towel to maintain humidity. Tubs were placed into constant temperature rooms set at 10; 15; 20; 25; 30; 35 and 40°C. The number of eggs in each Petri dish varied (3-10 eggs) as the number of eggs available from each overnight oviposition period was not consistent. Eggs were monitored daily until the first hatching, and twice a day thereafter until no further hatching occurred. Paper towel and filter paper were dampened as required. The mean number of days to hatch and total egg mortality were recorded at each temperature.

Differences in hatching times at each temperature were compared using a one-way ANOVA while lower developmental threshold (*t*) and the thermal constant *K* were determined using a major axis regression. Tukey HSD *post hoc* tests were used to determine between which temperatures significant differences occurred ($p \leq 0.05$).

2.2.2 Estimating feeding damage by *Neochetina eichhorniae* larvae

Newly hatched larvae from eggs reared at 25°C (following the methods set out in 2.2.1) were inserted into punctures, made with sterilised forceps, into the middle of leaf two and leaf three petioles of healthy water hyacinth plants. Each plant was inoculated with two larvae (one in each petiole) and kept in 10L of nutrient growth medium [2.8 mg/L N; 0.4 mg/L P] at 25°C. Control plants were not inoculated with larvae. Eight replicates of both control (no larvae) and treatment (with larvae) plants were used. Of these replicates, one control and one inoculated plant were sacrificed every seven days, for a period of 51 days. Sacrificed plants were drained, weighed and then dissected so that the larvae could be recovered. Larval recovery was used as a proxy for larval survival to calculate feeding rates each week. Plants were regularly inspected for the formation of pupal cases. Growth media were replaced weekly in order to maintain nutrient levels. All plants were grown under a 12:12 light to dark lighting regime. All plants had been cultured in nutrient solution for two weeks prior to the experiment. The experiment ran from mid-August to mid-October 2014 at the University of the Witwatersrand.

In order to determine the feeding rate per larva per day at 25°C, the below calculation was used,

$$Larval\ feeding\ rate = \frac{\left(\frac{total\ weight\ gains\ (all\ plants) - total\ weight\ losses\ (all\ plants)}{mean\ no.of\ larvae\ alive}\right)}{time}$$

(Equation 3)

The total plant weight gains and losses across all replicates at each temperature were used, and the mean number of larvae alive was estimated using the *average larval recovery rate* x *larvae remaining in the experiment*.

Differences in biomass change between control and larval treatments were compared using a General Linear Model (GLM) with time and treatment set as categorical predictors. Tukey HSD *post hoc* tests were used to determine where significant differences between control and larval treatment biomass change occurred.

2.2.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults

Twelve adult *Neochetina eichhorniae* weevils were each kept in temperature rooms at 15°C, 20°C, 25°C or 30°C for 24 hours. The sex of each weevil was determined before it was placed into a Petri dish with an excised water hyacinth leaf and moistened filter paper. Petri dishes were placed into 1.8L tubs lined with moistened paper towel to maintain humidity. Water hyacinth plants had been cultivated for a minimum of four weeks at high, medium and low nutrient concentrations using adaptations of Hoagland's macro solution (Hoagland and Arnon, 1950) in tap water, as specified in Table 2.1. Only leaves 2, 3, 4 and 5 on each plant were used, and were randomised within nutrient treatments. Leaves are numbered on a water hyacinth plant from youngest to oldest, where leaf 1 is the first fully unfurled leaf. Weevils were kept at a fixed temperature for three days with a 12h: 12h day: night cycle. Leaves were replaced and feeding scars were counted every 24 hours. Scars were classified as regularly shaped abrasions made on the leaf surface (Franceschini *et al.*, 2010). Holes through the leaf surface were counted as multiple scars as this only occurred where abrasions overlapped or aligned on opposite surfaces of the leaf (DeLoach and Cordo, 1976). Four replicates (two male and two female weevils per replicate) were used for each nutrient level at each temperature, totalling 48 weevils.

Differences between temperature and nutrient treatments as well as leaf number were compared using a General Linear Model. Where significant effects were found ($p \leq 0.05$), Tukey HSD *post hoc* tests were used to determine where they lay. The effect of weevil sex was not considered because of the small sample size (n=2 for each sex at each nutrient level at each temperature).

Table 2.1: Approximate concentrations of macronutrients (as specified by Hoagland and Arnon, 1950) in high, medium, and low nutrient solutions used to culture water hyacinth for temperature/nutrient feeding experiments.

Source	**Approximate concentrations (mg/L)**		
	High	**Medium**	**Low**
KH_2PO_4	0.76	0.19	0.00
KNO_3	1.80	1.39	0.07
$Ca(NO_3)_2.4H_2O$	3.60	1.39	0.07
$MgSO_4$	1.77	0.61	0.20
Tap water N	0.60	0.60	0.60
Tap water P	0.10	0.10	0.10
Final N	**6.00**	**2.00**	**0.67**
Final P	**0.86**	**0.29**	**0.10**
N:P Ratio	**6.98:1**	**6.90:1**	**6.70:1**

2.3 Results

2.3.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs

As expected, temperature significantly affected the period taken for the eggs to hatch ($F_{3, 97} = 4182$, $p < 0.01$). Hatching was only observed between 15°C and 30°C (Table 2.2). At 15°C, the mean hatch time was considerably longer as well as more variable than at higher temperatures. Using the reduced major axis method (Ikemoto and Takai, 2000), the thermal constant (K_E) and lower developmental threshold (t_E) were determined as 125.10°D and 11.95°C, respectively (Figure 2.1).

Table 2.2: The effect of temperature on the hatching time and % mortality of the *Neochetina eichhorniae* eggs at constant temperatures (n=225).

Temperature (°C)	n	No. of eggs hatched	Mortality (%)	Duration (days)	
				Range	Mean (± SD)
10	12	0	100	-	-
15	42	7	83	50-57	53.70 ± 2.81
20	43	33	23	13-17	15.30 ± 0.85
25	42	35	17	8-11	8.80 ± 0.67
30	43	26	40	6-9	7.50 ± 0.80
35	33	0	100	-	-
40	10	0	100	-	-

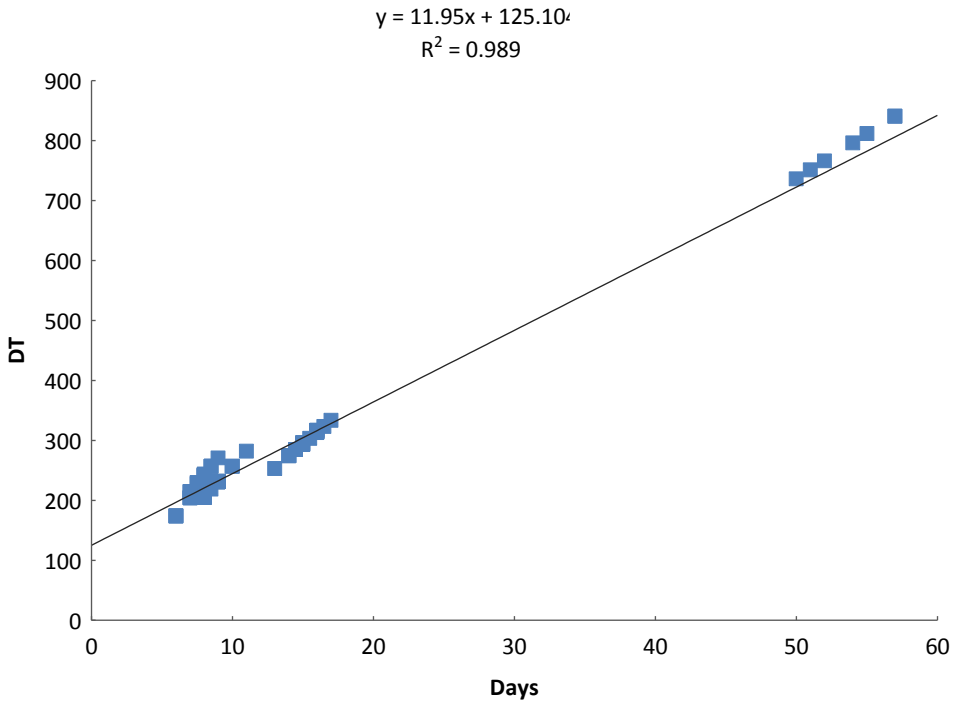


Figure 2.1: The effect of temperature on development of *Neochetina eichhorniae* eggs using the reduced major axis regression, where DT is the product of the egg duration and temperature (n=101; Ikemoto and Takai, 2000). The solid line represents the egg major axis regression $DT = t_E.D + K_E$.

During the experiment, 101 of the 225 eggs collected hatched successfully.

However, egg survival was not consistent across all temperatures. The proportion

of eggs hatching at a given temperature, decreased significantly below 20°C (Figure 2.2). Experimental temperatures that did not fall between 15°C and 30°C were considered to lie off the survival curve and were not included in the regression for S_E, which produced an R^2 value of 0.99.

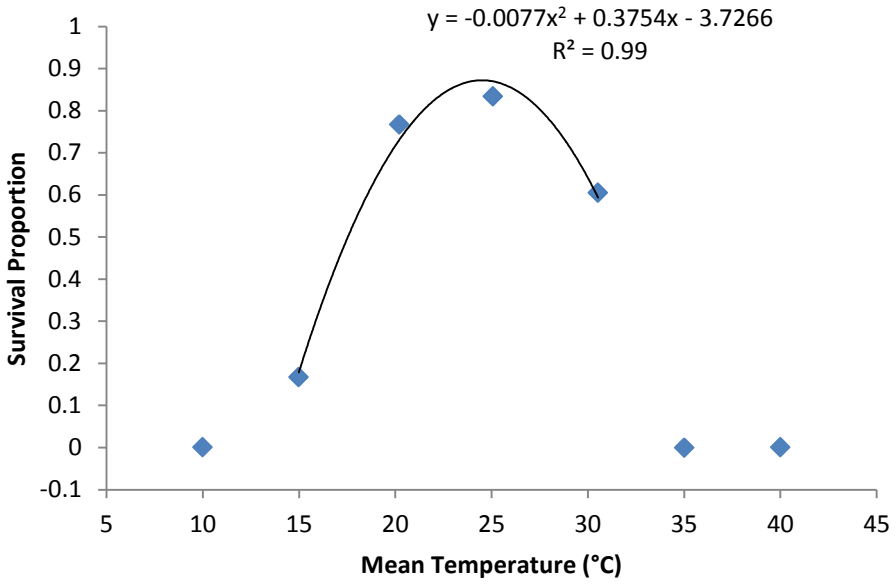


Figure 2.2: The effect of temperature on the survival of *Neochetina eichhorniae* eggs (n=225). The solid line represents the second order polynomial regression between 15-30°C. Temperatures where no hatches occurred were not included in the regression.

2.3.2 Estimating feeding damage by *Neochetina eichhorniae* larvae

Overall, *N. eichhorniae* larval feeding on treatment plants resulted in a 52% biomass loss by day 51 (Figure 2.3). Differences in biomass change between control and treatment plants were significant for all weeks ($F_{6, 56} = 10.48$, $p < 0.01$). On day 29, the biomass gain in the control plants decreased slightly. This is likely because of an outlier, a small plant that suffered high biomass loss over the course of the experiment. Removing this outlier does not change the nature of the relationship between control and treatment plants, which remains significantly different ($F_{6, 52} = 12.95$, $p < 0.01$).

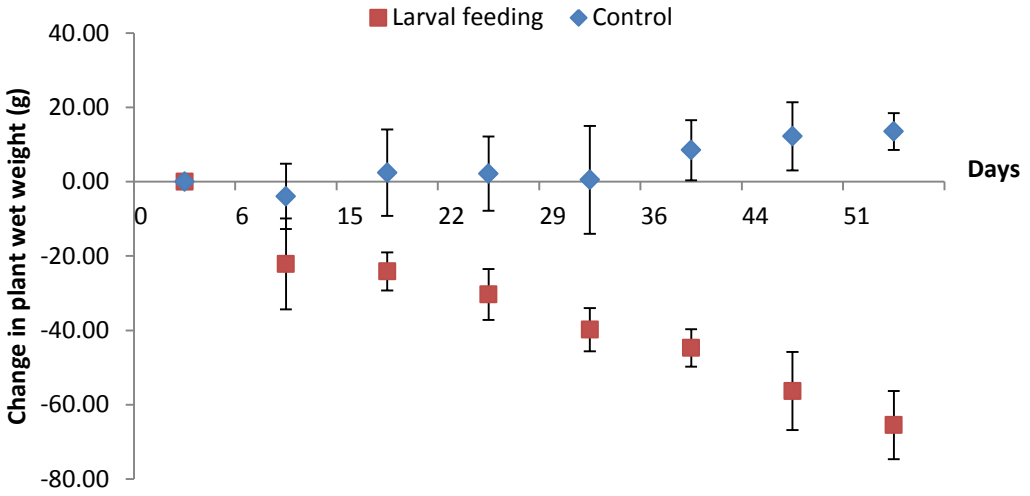


Figure 2.3: Effect of larval feeding by *Neochetina eichhorniae* on water hyacinth plant biomass (wet weight, g) at 25°C over 51 days. Plants were grown in 2.8 mg/L N and 0.4 mg/L P nutrient solutions at 25°C and were destructively sampled each week (n=2-8 per treatment per week, means ± SD). Plant biomass each week was compared to the initial biomass.

Estimated feeding rates per larva were calculated each week using an average larval recovery rate of 0.60 larvae/plant/week. In the first week, an artefact appears, possibly resulting from the general plant decline in the first 6 days of experimentation. Larval feeding rates in the remaining days of the experiment are relatively constant, fluctuating around a mean of 0.90 g/larva/day (Figure 2.4).

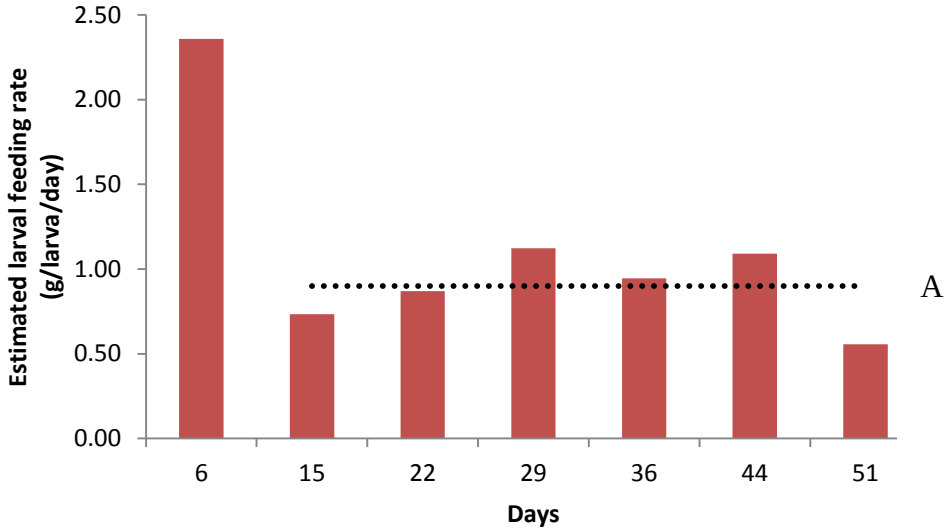


Figure 2.4: Biomass removal by *Neochetina eichhornaie* larvae over 51 days using estimates of larval numbers. Plants were grown in 2.80 mg/L N and 0.40 mg/L P nutrient solutions at 25°C. The line A indicates a mean of 0.90 g/larva/day between day 15 and day 51.

2.3.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults

Data have been presented in several formats to tease out the relationships between variables. The effect of temperature alone ($F_{3, 34} = 47.54$, $p < 0.01$; Figure 2.5) and the effect of leaf number alone ($F_{3, 85} = 7.72$, $p < 0.001$; Figure 2.6) were both significant, with adult feeding rates increasing with increasing temperature and decreasing leaf age, respectively. However, the effect of nutrients alone on adult feeding rate was not significant ($F_{2, 34} = 0.62$, $p > 0.05$). Interaction effects between temperature and nutrients ($F_{6, 85} = 0.80$, $p > 0.50$; Figure 2.7), temperature and leaf number ($F_{9, 85} = 0.72$, $p > 0.60$), nutrients and leaf number ($F_{6, 85} = 0.83$, $p > 0.50$), and between temperature, nutrients and leaf number were not significant ($F_{18, 85} = 0.59$, $p > 0.80$;
Figure 2.8).

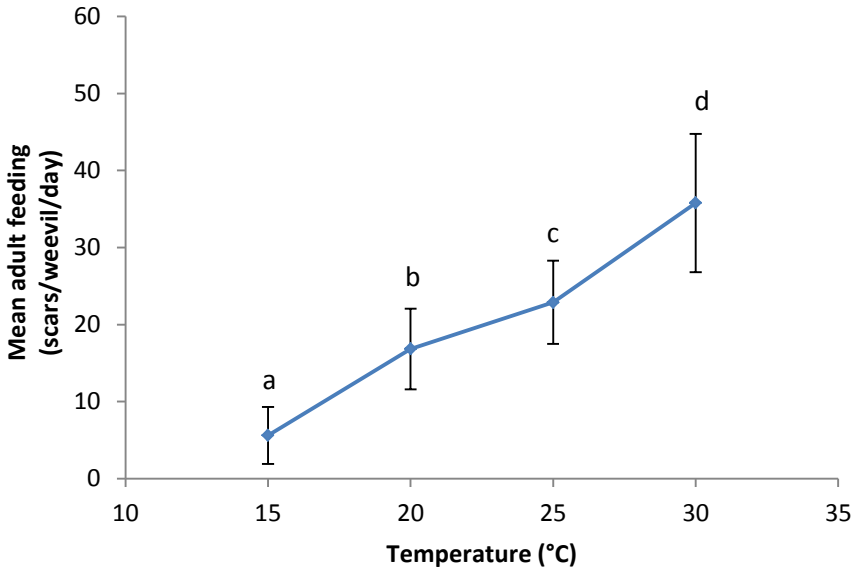


Figure 2.5: The effect of temperature on *Neochetina eichhorniae* adult feeding rates (means ± SD). Different nutrient and leaf age treatments have been combined for each temperature treatment. Significant differences between means are indicated by different letters.

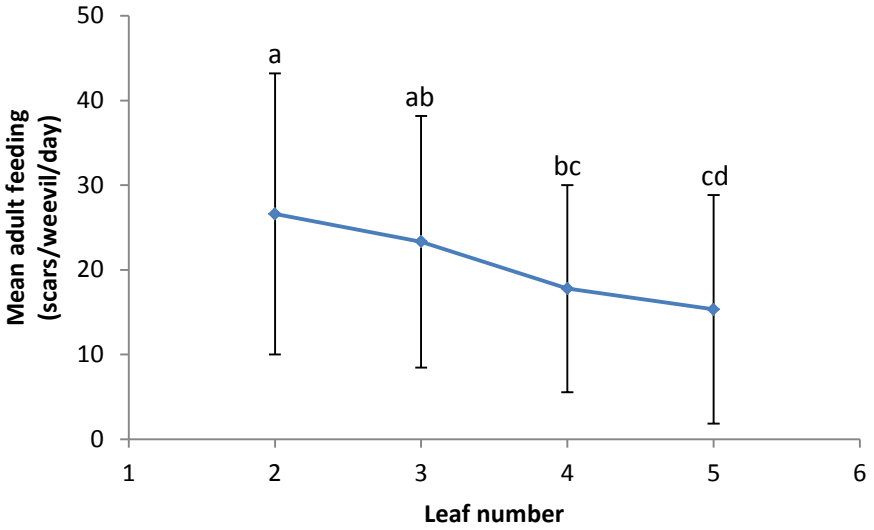


Figure 2.6: The effect of leaf age on *Neochetina eichhorniae* adult feeding rates (means ± SD). Lower leaf numbers correspond to younger leaves. Nutrient and temperature treatments were combined per leaf. Different temperature treatments have been combined for each leaf age treatment. Significant differences between means are indicated by different letters.

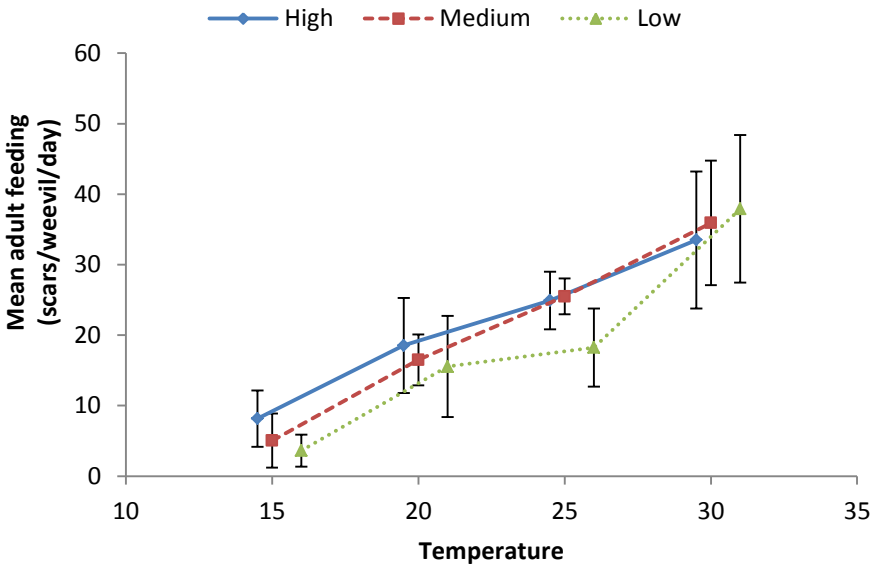


Figure 2.7: The effect of nutrients on *Neochetina eichhorniae* adult feeding rates for plants grown at high [ca. 6.00 mg/L N; 0.86 mg/L P], medium [2.00 mg/L N; 0.29 mg/L P], and low [0.67 mg/L N; 0.10 mg/L P] nutrients at increasing temperatures (means ± SD). Different leaf numbers were combined to determine an overall temperature*nutrient interaction.

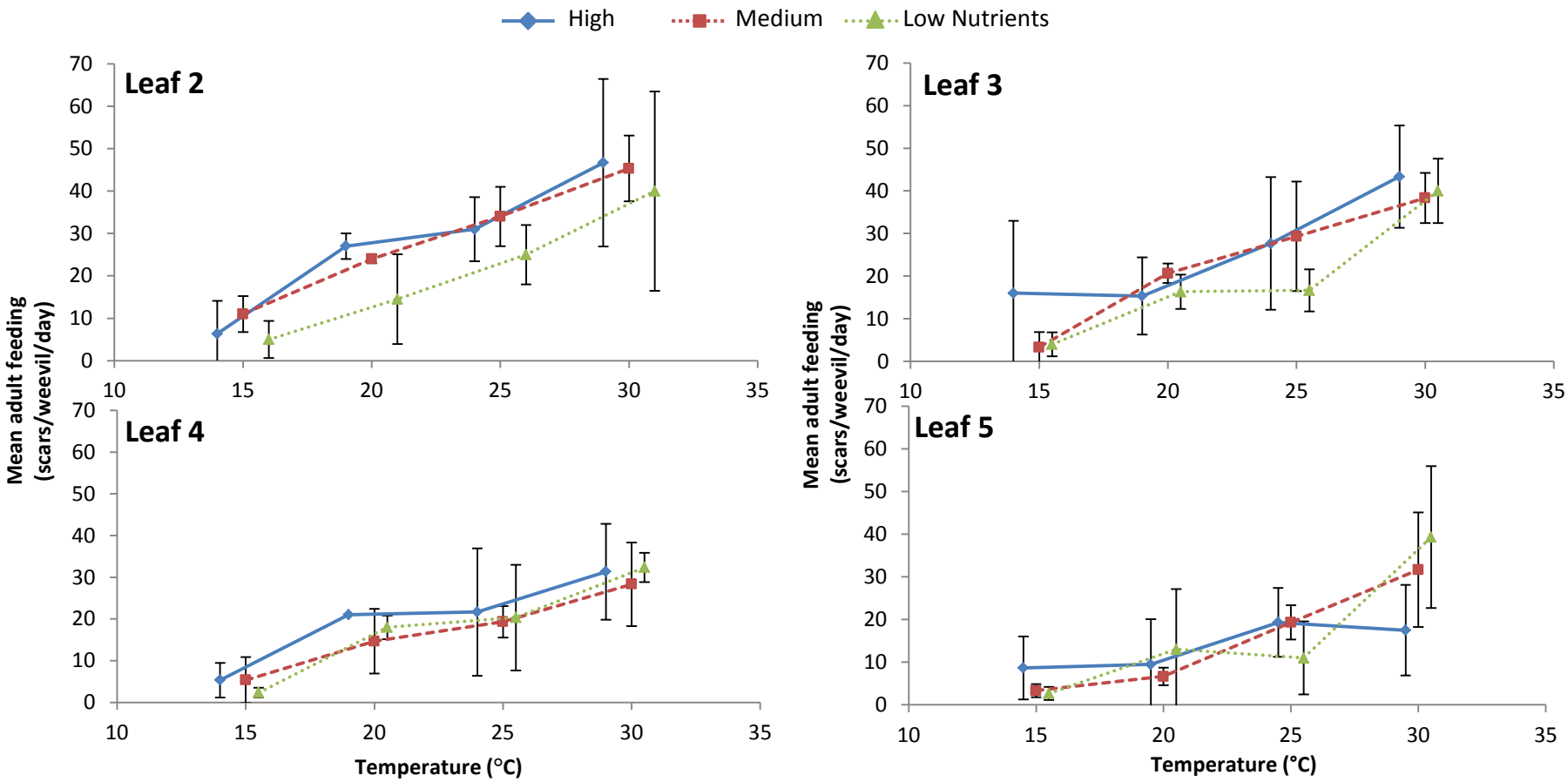


Figure 2.8: The effect of temperature on *Neochetina eichhorniae* adult feeding rates on water hyacinth plants grown at high [ca. 6.00 mg/L N; 0.86 mg/L P], medium [2.00 mg/L N; 0.29 mg/L P], and low [0.67 mg/L N; 0.10 mg/L P] nutrients (means ± SD). Both high and low nutrient values have been slightly offset for ease of graph interpretation.

2.4 Discussion

2.4.1 Temperature-dependent development and survival of *Neochetina eichhorniae* eggs

Fastest hatching times (Table 2.2) correspond with the literature (DeLoach and Cordo, 1976; Stark and Goyer, 1983) but hatching at low temperatures does not. Some literature suggests that eggs of *N. eichhorniae* do not to hatch below 20°C (DeLoach and Cordo, 1976; Julien, 2001), and King (2011) suggests that the lower developmental threshold (*t*) of *N. eichhorniae* is approximately 15.20°C. In this study, hatching occurred at a mean temperature of 14.98°C, and *t* was calculated as 11.95°C. Hatching at this temperature, however, takes 50 to 57 days with a 17% survival rate. While water hyacinth leaves are known to live as long as 101 days in Florida (Center, 1980), *Neochetina* weevils prefer to oviposit in intermediate (leaf position 4; ca. 20-64 days old) to old aged leaves (leaf position 6; ca. 38-96 days old; Center, 1987). Should eggs take 50 to 57 days to develop and hatch these older leaves will have begun to senesce, probably resulting in 100% mortality of the eggs that are laid late in the season. Increased development time and decreased survival can result in decreased rates of insect population increase, and may contribute the slow establishment of some agent populations (McClay and Hughes, 1995), and decreased levels of control. Successes in the control of water hyacinth infestations have occurred in Bangalore, India (Jayanth, 1988) and on Lake Victoria (Wilson *et al.*, 2007) but average minimum temperatures for Bangalore and Entebbe are above 17°C and 19°C, respectively (Jayanth, 1988; Weatherspark, 2015). Weevil populations are thus able to develop faster and are likely to have higher survival rates, leading to faster population increases.

K and *t* were estimated as 125.10°D and 11.95°C respectively (Figure 2.1). This suggests that the lower developmental threshold for *N. eichhorniae* eggs is somewhat lower than previously estimated by King (2011) from compiled data (15.20°C), but similar to his estimates from combined species data (both *N. eichhorniae* and *N. bruchi*; 11.40°C). The thermal constant, however, was

estimated at 94.70°D (compiled data) and 122°D (combined data) by King (2011). Discrepancies in *K* may result in significantly different estimations of generations or population densities. This is because degree-day (°D) values are used to quantify the duration of insect development by summing the number of heat units that occur above *t* (Wagner *et al.,* 1984). Differences in the value of *K* would lead to different developmental durations under the same temperature conditions, impacting population and generation estimations. Generation estimates for two sites in South Africa (Delta Park and Mbozambo Swamp), were 2.19 and 4.39 generations per year respectively (King, 2011). However, if the estimate of *K* for egg development found here is included, estimated generations at each site drop to 1.60 and 3.21 generations per year, respectively. Fewer generations per year results in slower population growth and potentially decreased levels of water hyacinth control.

A significant temperature-dependent egg survival relationship was found, with egg survival proportions ranging from 0.17 (at 15°C) to 0.83 (at 25°C; Figure 2.2). However, DeLoach and Cordo (1976) found that egg survival proportions ranged from 0.00 to 0.71 at the same temperatures. Interestingly, at 30°C and 35°C survival proportions observed by DeLoach and Cordo (1976) were 0.68 and 0.55, while in this study they were only 0.60 and 0.00, respectively. Weevils used by DeLoach and Cordo (1976) were collected at Campana, Argentina (1971-1974) while those used in this study were mass-reared by the South African Sugarcane Research Institute (SASRI) in KwaZulu Natal, South Africa (2013-2014). Any differences in survival and development might result from selection for lower temperatures in the South African populations (Gillespie *et al.,* 2004). *Neochetina eichhorniae* was released in South Africa in 1974, so even at cold sites such as Delta Park, populations are likely to have gone through up to 88 generations and up to 175 generations at warms sites like Mbozambo Swamp (using data from Byrne *et al.,* 2010). Differences in insect species characteristics can occur in as little as 10 generations (Mohaghegh *et al.,* 1999) suggesting that the environment occupied by water hyacinth populations in South Africa have selected for Neochetina populations adapted to such conditions.

In this study, maximum egg survival was at 25°C but development was fastest at 30°C. Similarly, DeLoach and Cordo (1976) showed maximum egg survival at 25°C, but fastest development at 35°C and 30°C for *N. eichhorniae* and *N. bruchi* respectively. Rueda *et al.*, (1990), Liu *et al.,* (2002) and Goebel (2006) also found differences in maximum survival and fastest development times, while Shima and Hirose (2002) found maximum development and survival occurred at 27.50°C. It is possible, then, that the optimal temperature for development and survival of *N. eichhorniae* eggs may lie between 25°C and 30°C.

However, over and above the effects of temperature on insect egg survival, predation and stochastic effects such as frost are also likely to influence survival. Generalist predators can disrupt biological control systems (Snyder and Ives, 2001) and *Neochetina* weevils are exposed to predators and parasites in Argentina (DeLoach and Cordo, 1982) and Louisiana (Stark and Goyer, 1983). Although little is known about such factors in South Africa, predation and parasitism can lower survival rates of *Neochetina* weevils. Additionally, at colder sites weevils, particularly the egg populations, are exposed to up to 101 frost days per year (Byrne *et al.,* 2010), which result in damage to water hyacinth leaves and petioles (King, 2011) further reducing potential survival of *Neochetina* eggs and larvae.

Understanding insect development and survival rates is important when trying to estimate population growth in the field, as stage-specific survival influences population growth rates by altering population densities (Birch, 1948). However, estimates made in the laboratory may reflect the maximum possible "hatchability" of eggs, but do not reflect actual egg survival in the field, as eggs in the lab do not develop *in situ* and leaf senescence, frost, and potential predation and parasitism is not accounted for.

2.4.2 Estimating feeding damage by *Neochetina eichhorniae* larvae

The impact of *Neochetina* larval feeding on water hyacinth plant growth was significant. Specific *N. eichhorniae* larval feeding rates have not been measured before, but Akbay *et al.,* (1991) and Wilson (2002) both used the assumption that

third instar *Neochetina* larvae consume the *equivalent* of approximately 0.18 leaves/larva/day, even though larvae do not feed on the leaf lamina. Using Wilson's (2002) assumptions of leaf fresh weights between 4-20g would result in larval feeding rates of 0.72-3.60 g/larva/day. In this study, larval feeding rates were calculated each week at 25°C, and were estimated at an average of 0.90 g/larva/day (excluding the first week). This feeding rate falls well within the range of expected larval feeding rates proposed by Akbay *et al.,* (1991) and Wilson (2002), and was calculated using empirical data of fresh plant weights.

Although 25°C may not be the optimal feeding temperature, evidence shows that it is the optimal temperature for *Neochetina* survival (DeLoach and Cordo, 1976; Section 2.3.1), which is vital for determining feeding from small larval numbers. Increasing temperature increases metabolic reactions and increases energy requirements in insects (Wigglesworth, 1974), meaning that the estimated larval feeding rate of 0.90 g/larva/day will likely change with changing temperatures. Adult *Neochetina* feeding is also temperature-dependent and generally reaches a maximum rate at approximately 30°C (Shih *et al.*, 1994; Chikwenhere, 2000; Jianhao *et al.*, 2003). An estimate of 0.90 g/larva/day is therefore unlikely to represent the maximum larval feeding rate, but serves well as an initial estimate for larval biomass removal. If larval feeding occurs at a rate of 0.90 g/larva/day, total consumption per larva during the 50-day developmental period (at 25°C; DeLoach and Cordo, 1976) would be 45 g/larva, approximately three times the amount control plants are able to grow under the same conditions. Larval feeding is therefore extremely damaging to water hyacinth plants, but it will likely change with variable temperature and nutrients conditions, altering the impact that larvae have on water hyacinth populations under different climatic regimes.

2.4.3 Temperature and nutrient-dependent feeding by *Neochetina eichhorniae* adults

Temperature

The effects of temperature on insect feeding are well-documented (Del Fosse, 1977; El Abjar and Bashir, 1984; Ferro *et al.*, 1985; Lactin and Johnson, 1995). In most cases, feeding rate increases significantly with increasing temperatures. Feeding by adult *Neochetina eichhorniae* weevils is no different. An increase of 10°C between 20-30°C approximately doubles the adult feeding rate, in accordance with the Q_{10} Rule (Figure 2.5; Van't Hoff, 1898; Sato, 1988). Weevils therefore consume more leaf biomass at higher temperatures to cope with increasing activity and energy demands. However, Mathavan and Pandian (1975) and Bauerfeind and Fischer (2013) show that while increased temperatures can increase insect feeding rates and overall consumption, food conversion efficiencies are reduced. This suggests that insects increase their feeding rates to compensate for decreased food conversion efficiencies so that energy demands are still met. Regardless of how insects utilize their food, at sites with warmer temperature regimes, *Neochetina* weevils are expected to remove greater water hyacinth biomass, because of increased feeding rates, and may cause increased damage and control of water hyacinth infestations.

Leaf Age

Water hyacinth leaf number and hence the relative age of water hyacinth leaves also significantly affected the rate at which adult weevils fed (Figure 2.6), with leaf 2 feeding rates being significantly different to both leaf 4 and leaf 5. Center and Wright (1991) suggest that high levels of natural plant products, such as phenolic compounds in younger leaves attracts and stimulates weevil feeding resulting in high adult feeding rates on younger water hyacinth leaves. Other studies showing that *Neochetina* weevils preferentially feed on younger leaves have attributed this to differential nutrient quality between leaf ages, with younger leaves having higher nitrogen, phosphorus and potassium contents (Center, 1984; Center and Wright, 1991; Dray *et al.*, 2012). Although Mattson (1980) suggests that increased plant quality may reduce feeding rates, Levesque *et al.*, (2002)

showed that increasing plant quality further increases insect feeding rates. Additionally, increased plant quality directly affects weevil reproductive capacities (Buckingham and Passoa, 1984; Center and Dray, 1992; Center and Dray, 2010*a*); hence, preferential feeding on younger, nutrient-rich foliage by females would increase their potential contribution to the next (F_1) generation.

Nutrients

Although temperature significantly influenced the rates at which adult weevils made scars, these rates were not influenced by the different nutrient levels. While increases in nutrient concentrations have been found to increase leaf tissue consumed by other biocontrol agents, such as adult *Mogulones cruciger* (Coleoptera: Curculionidae) on houndstongue (van Hezewijk *et al.*, 2008), adult feeding by *Neochetina* weevils seems not to be affected. Both Heard and Winterton (2000) and Coetzee *et al.* (2007*a*) showed that nutrients did not significantly affect the number of scars made by adult *Neochetina* weevils or feeding damage by *Eccritotarsus catarinensis* (Heteroptera: Miridae) respectively. However, in both of these studies, plant growth was significantly affected. Total biomass, ramet production and petiole lengths were significantly higher at higher nutrients (Heard and Winterton, 2000). Host plant nutrient quality therefore may not feedback into herbivore populations through direct effects on adult herbivory.

Plant nutrients and quality have been shown to affect weevil reproductive capacity greatly by altering follicle and ovary capacities (Center and Dray, 1992; Center and Dray, 2010*a*). As nutrients increase, females become more reproductively active, resulting in more eggs and subsequently larger larval populations, which cause majority of the damage to water hyacinth plants. Larval insect feeding has been shown to change with changing nutrient concentrations (Sands *et al.*, 1983; Canavan *et al.*, 2014). Additionally, insect larval survival, size and development rates are also influenced by nutrient concentrations (Lindroth *et al.*, 1997; Kingsolver and Woods, 1998; Paritsis and Veblen, 2010). It is likely then that increased control at higher nutrients is a compound effect of more larvae, which have developed faster and larger from larger weevil populations rather than

increased herbivory by adult weevils. However, plant growth is also increased at high nutrients (Reddy *et al.*, 1989; Reddy *et al.*, 1990) and while agent populations may benefit from increased plant quality, plants may be able to compensate for the effects of herbivory as a result of high growth rates, as seen with *Eccritotarsus catarinensis* on water hyacinth (Coetzee *et al.*, 2007*a*)

Interactions

*Temperature*Nutrients* – The interactions of insect diet with temperature have been generally considered (Forno and Bourne, 1985; Stamp and Bowers, 1990; Lindroth *et al.*, 1997). Most often, the effect on insect growth rate or stage duration is considered, but consumption rates are just as important for understanding insect herbivory in terms of biomass removal. Some investigations show that interactions between the effects of temperature and the effects of plant quality can significantly influence insect feeding (Kingsolver and Woods, 1998; Lee and Roh, 2010), but such was not the case in this study. Although not significant, feeding rates on low nutrient plants were the highest of all nutrient treatments at 30°C (Figure 2.7). Kingsolver and Woods (1998) showed a similar trend in *Manduca sexta* (Lepidoptera: Sphingidae) caterpillars, where higher consumption rates occurred on low protein diets between 18-34°C. Mattson (1980) suggests that organisms with low nitrogen availability must consume more food to meet their nitrogen demands, while higher temperatures increase metabolic and energy requirements (Wigglesworth, 1974) and may further increase insect consumption rates. However, the lack of a significant interaction between diet and temperature for *N. eichhorniae* suggests that perhaps temperature is more important in determining adult *Neochetina* feeding, similar to *Cyrtobagous salviniae* (Coleoptera: Curculionidae) on the invasive aquatic plant, *Salvinia molesta* (Forno and Bourne, 1985).

Furthermore, plant quality changes under different temperatures (Bauerfeind and Fischer, 2013). While all plants in this study were grown under the same initial temperature conditions, the plant nutrients were never quantified. Plant nutrients

may not have varied significantly between nutrient treatments to result in differential adult herbivory at changing temperatures.

*Temperature*Nutrient*Leaf Age* – Individually, temperature (DeLoach and Cordo, 1976; Shih *et al.*, 1994), nutrients (Heard and Winterton, 2000), and leaf age (Center and Wright, 1991) are known to influence *Neochetina* weevil populations, but their combined effects on adult weevil herbivory were previously unknown. Here the interaction of these factors was tested and was found not to be a significant influence (Figure 2.8). Although both temperature and leaf age influence adult feeding rates, nutrients and nutrient interactions do not appear to do so. Nutrients, particularly nitrogen, are known to affect insect herbivory (Mattson, 1980; Levesque *et al.*, 2002) but generally do not significantly influence the feeding of some water hyacinth biological control agents (Heard and Winterton, 2000; Coetzee *et al.*, 2007*a*). Many insects undergo maturation feeding, which is required for reproductive development (Strong, 1967; Wainhouse *et al.*, 2007), needing to consume food to reach a particular size in order to initiate reproductive processes. Consuming food that is more nutritious would thus result in shorter maturation periods and subsequently increased population growth. However, *Neochetina* weevils oviposit from the first day of eclosion (DeLoach and Cordo, 1976) and do not undergo maturation feeding. Weevils therefore do not require nutritious food to initiate reproduction, though food quality may influence their reproductive capacity (Center and Dray, 1992; Center and Dray, 2010*a*).

Ripley *et al.*, (2008) found that biomass removal by weevils reduced photosynthetic rates of water hyacinth far more than an equivalent artificial biomass removal through leaf area excision. Venter *et al.*, (2013) explored this difference and found that weevil-borne microbes contribute as much to the reduction in photosynthesis as does herbivory. Adult weevil feeding therefore encompasses more than just tissue consumption by the adult weevils and a maximum removal rate of 86.30 mm^2 /weevil/day (DeLoach and Cordo, 1976), which equates to ~0.015 g/weevil/day, may not be as trivial as it seems. The use

of biomass removal alone in determining the impact of *Neochetina* weevils on water hyacinth populations is therefore likely to underestimate the effect of biological control.

2.5 Conclusion

Temperature is particularly important in determining insect development, survival and feeding rates, which in turn influence the number of generations, population densities and potential level of control that can occur at a specific site. Calculated *Neochetina eichhorniae* egg lower thermal thresholds (*t*), degree-day requirements (*K*) and temperature-dependent survival proportions provide insight into egg population dynamics and they can be used to model weevil populations, in conjunction with larval and pupal values of *t* and *K* from the literature.

Although larval feeding has not received enough attention in the literature initial estimates of biomass removal (0.90 g/larva/day) have been made here. However, specific feeding rates and effects on plant growth parameters, particularly under different temperature and nutrient regimes still need to be investigated. These calculations will be both interesting and enlightening, helping researchers to understand how the most damaging life-stage of *Neochetina* weevils affects water hyacinth populations. Quantifying larval feeding effects will be critical to modelling interacting populations of water hyacinth and *Neochetina* weevils accurately. Furthermore, the relationship between weevil feeding, temperature and nutrients appears complicated with temperature but not nutrients affecting specific feeding rates. Nutrients, however, influence both the survival and the growth of *Neochetina* populations as well as water hyacinth populations, and may therefore need to be considered when estimating these populations.

Experimentally determined values for egg lower thermal thresholds (*t*), egg degree-day requirements (*K*) and weevil larval feeding, as well as temperature-dependent relationships of egg survival and adult weevil feeding have been incorporated into models of *Neochetina eichhorniae* biological control of water hyacinth in Chapter 3.

3 CHAPTER THREE – CONSTRUCTING A MODEL OF WATER HYACINTH BIOLOGICAL CONTROL

3.1 Introduction

3.1.1 Types and uses of models in biological control

Models are used extensively in ecology and agriculture to model complex systems because of their ability to simulate and predict outcomes, deal with ecological complexity and increase the understanding of the systems under investigation (Freckleton and Stephens, 2009; Mills and Kean, 2010). A wide variety of models exists, ranging from conceptual ecological models used for planning (Ogden *et al.*, 2005), to mathematical and population dynamic models used in invasion biology and biological control (Grundy, 2003; Rafikov *et al.*, 2008; Kriticos *et al.*, 2009). In weed ecology, specifically the biological control of weeds, models have become increasingly important, being used throughout the different phases of biological control programmes, for different purposes (Table 3.1).

Table 3.1: Applications of modelling in biological control systems

Agent	Weed	Both
• Select new and more effective agents[3,7,8,10,14]	• Predict weed emergence[5]	• Predict distributions[11]
• Estimate agent effectiveness[1,12]	• Predict plant invasion dynamics[4]	• Cost-benefit analyses[13]
• Determine climatic requirements of insects[11]	• Invasion risk analysis[15]	• Post-release evaluation[9]
• Determining optimal agent release population size[14]	• Determine targetable weed lifestages[2,3,14]	• Improve understanding of biological control success[14]
• Inform control and release strategies[13,14]		
• Non-target risk analysis[6]		

1) Rees and Paynter (1997); 2) Shea and Kelly (1998); 3) McEvoy and Coombs (1999); 4) Parker (2000); 5) Grundy (2003); 6) Andersen *et al.*, (2005); 7) McClay and Balciunas (2005); 8) Senaratne *et al.*, (2006); 9) Sims *et al.*, 2006; 10) van Klinken and Raghu (2006); 11) Zalucki and van Klinken (2006); 12) Kriticos *et al.*, (2009); 13) Morin *et al.*, (2009); 14) Mills and Kean (2010); 15) Robinet *et al.*, (2012)

Although numerous types of models exist, process-based and niche models are favoured in biological control. Niche models rely on the theoretical relationships between observed distributions of species and environmental predictors (Morin and Thuiller, 2009). These models are generally used in 'climate-matching' between species native range and invaded or introduced range, using meteorological values to determine the 'climate envelope' (Zalucki and van Klinken, 2006). These climate envelopes are then used to determine the potential distributions of plants and insects globally. Programmes such as CLIMEX are frequently used in biological control to determine potential spatial and temporal distributions of biocontrol agents (Coetzee *et al.*, 2007*b*; Lawson *et al.*, 2008). While these models serve as useful tools, they do not typically consider organism phenology (timing of developmental events), specific insect life stages or population size (Aurambout *et al.*, 2009). As such, process-based models may be considered more useful.

In contrast to climate-matching models, process-based models incorporate explicit biological processes and they can be used to predict abundance, cover and even phenology of organisms by including multiple life stages and life history characteristics (Aurambout *et al.*, 2009; Morin and Thuiller, 2009). Such process-based models are generally more complex than CLIMEX models and require specific data on species traits, such as developmental rates. Software, such as STELLA and DYMEX, which use modular components on a graphical interface, make model-building and modification user friendly and make models easier to understand (Aurambout *et al.*, 2009; Kriticos *et al.*, 2009). Process-based models can be constructed to simulate whatever biological process is of interest, given data or information for that process exists.

For the purposes of biological control, population models are often of the most interest as it is important to understand and demonstrate how biocontrol agents affect the target weed, not only at the individual level, but also at the population level (Kriticos *et al.*, 1999). Process-based population models can be built to predict how these weeds and agents interact at the population level. However, for

these models to be constructed and parameterised, a sound knowledge of population dynamics and life history characteristics is needed.

3.1.2 Model population dynamics and parameterisation

All populations are dynamic in time and can be characterised by specific factors, such as temperature and nutrients. Incorporating these important factors into population models can therefore result in accurate population estimation and evaluation, which is imperative in determining the effect of biological control agents.

The occurrence of macrophyte populations is determined by abiotic parameters such as light, temperature, nutrients, water movements and disturbances (Bornette and Puijalon, 2011). Populations of water hyacinth, however, are limited by temperature, nutrients, natural enemies, salinity, and disturbance (Wilson *et al.*, 2001). Of these limiting factors, temperature and water nutrients are considered the major determinants (Lorber *et al.*, 1984; Wilson *et al.*, 2001; Wilson *et al.*, 2005; Byrne *et al.*, 2010). Water hyacinth density further influences its population growth (Reddy and DeBusk, 1984; Sato, 1988). Although water nutrients are known to stimulate plant growth (Reddy *et al.*, 1989; Reddy *et al.*, 1990), relatively little is known about how temperature and nutrients interact to influence water hyacinth growth (Sato, 1988) and density-dependence. As such, the main parameters considered in modelling water hyacinth populations in this study are temperature and population density. Plant population density is further affected by the herbivory of agent populations, the level of which is determined by *Neochetina eichhorniae* population densities.

Although *N. eichhorniae* populations are highly dependent on water hyacinth populations, the use of only Lotka-Volterra equations to determine population densities is not completely appropriate, as these equations do not incorporate insect development and timing, which is important when multiple life stages influence the plant. A stage-structured approach to modelling insect development and population estimation has therefore been taken in this study.

Insect development is greatly influenced by the prevailing temperatures (Higley and Haskell, 2002). Insects only develop above a certain temperature (*t*), accumulating heat units or degree-days until they reach a specific thermal threshold (*K*) (Ikemoto and Takai, 2000), thereby completing a developmental phase and allowing them to move into the next life stage. Environmental temperatures therefore determine how long each life stage will take to develop, and when individuals will move between life stages. However, being able to model insect development still does not solely determine insect population densities.

The growth of insect populations is determined by the capacity of that population to survive and reproduce (Birch, 1948). Adult oviposition and life stage survival is therefore of the utmost importance in determining *Neochetina eichhorniae* population densities, and are both temperature-dependent (DeLoach and Cordo, 1976; Section 2.3.1). As mentioned above, the weevil populations influence the water hyacinth population through herbivory. Feeding rates and biomass removal by both larval and adult weevil populations consequently need to be included in any model of water hyacinth biological control.

In this chapter, a process-based population model of water hyacinth biological control is constructed. Although nutrient relationships within both species populations remain important, only temperature has been incorporated as the determining environmental factor.

3.2 Methods

3.2.1 Model construction

Model construction and parameterisation is an iterative process, and as such, various steps were repeatedly taken in order to develop a realistic, functioning model. The model was constructed in STELLA (isee Systems, New Hampshire) modelling software. The interaction of model parameters was first described visually in model maps using standard systems modelling notation consisting of

"stocks", "flows", and "converters" (Figure 3.1). Stocks function as points of accumulation or reservoirs (e.g. population size/density) and are represented as rectangles, while flows function as a change to the stock increasing (positive flow) or decreasing (negative flow) the amount accumulated. Flows are represented by an arrow with a valve. The direction of an arrow, relative to the stock, indicates whether a flow is positive or negative. Converters are represented by pink arrows and functions as any defined variable that alters a flow. Please note that in these models an interval of simulation time occurs between calculations, known as Delta time (*dt*). The value of *dt* determines how often in each time step model values are recalculated. In all models presented here, *dt* is set to one. Numerical values in these models are therefore recalculated on a daily basis.

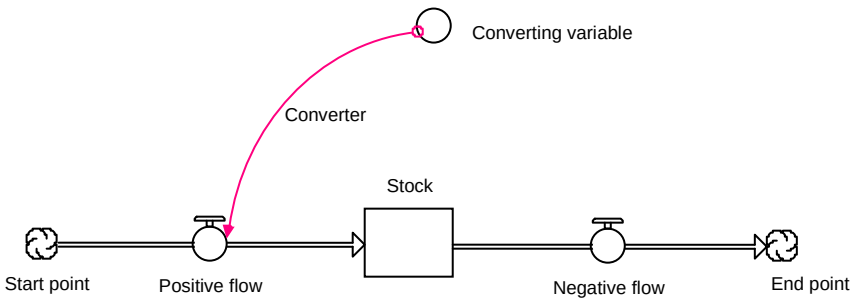


Figure 3.1: Illustration of systems modelling notation in STELLA. Direction of flow is determined by the direction of the arrowhead. Positive flows are directed into the stock, while negative flows are directed away from the stock.

Once model maps were created in STELLA (e.g. Figure 3.2) they were defined mathematically using relationships derived from empirical data from temperature-dependent experiments on water hyacinth and *Neochetina* weevils. Where the literature contained good data for model parameters, these values were used in the model. All model variables can be found in the Appendix.

Water hyacinth and weevil populations were first modelled independently. Numerous versions of each model were created, but only important stages have been reported here. Stage 1 water hyacinth models were simple models that

excluded the effects of temperature. These models were then developed into Stage 2 water hyacinth models that incorporated variable temperature. Weevil models were developed in three model stages. Life stages were systematically added until the full life cycle of the weevil was present in the model structures. Stage 1 weevil models included variable temperature with constant oviposition (based on mean site temperatures) and 100% survival between life stages. These models were developed into Stage 2 weevil models that incorporated variable temperature with temperature-dependent oviposition and temperature-dependent survival. Stage 3 weevil models then incorporated age-dependent oviposition as well as stage-dependent winter mortality.

Water hyacinth and weevil models were then integrated through the effect of herbivory on the weed. It is important to note that although the modelled weevil populations affect modelled water hyacinth populations through herbivory, modelled water hyacinth populations do not in turn affect weevil population models. Bottom-up effects on weevil populations occur through plant nutrient quality (Center and Dray, 2010*a*), which has not been included in the models thus far. Weevil population models are thus only influenced by temperature, operating independently.

Model variables have been listed in the Appendix. All models were run for a period of two years, for two sites in South Africa, Mbozambo Swamp (29°21'S, 31°18'E), and Delta Park (26°07'S, 28°00'E), which are representative of the warmest and coldest sites sampled in Byrne *et al.* (2010). All temperature data has been drawn from Byrne *et al.* (2010).

3.2.2 Modelling water hyacinth

Logistic growth of water hyacinth

Growth of water hyacinth is accurately described by the logistic growth equation (Gutiérrez *et al.*, 2001; Wilson *et al.*, 2001; Wilson *et al.*, 2005), and has been used to describe the water hyacinth biomass density term of V (kg/m^2). The model

map, (Figure 3.2) was described using the logistic growth equation, which is density-dependent.

$$\frac{dV}{dt} = r_V V(1 - \frac{V}{K})$$

(Equation 4)

The two parameters in this model are the intrinsic growth rate of water hyacinth r_v (1/day), and the carrying capacity of the system K (kg/m^2). The intrinsic growth rate describes the maximum physiological rate of biomass growth in the absence of competition for resources (Otto and Day, 2007). Although the realised biomass growth rate is often different to the intrinsic growth rate of biomass, water hyacinth is often in environments where there are no other competitors for resources. As such, the intrinsic growth rate used in the Stage 1 (excluding temperature) model has therefore been assumed to approximate a realistic water hyacinth biomass growth rate, at a value of 0.052 (g/g/day) (Center *et al.*, 1982; Wilson *et al.*, 2005).

The carrying capacity of a system is the maximum biomass per unit area of a species that an area can support without reducing its ability to support the same species in the future (Maler, 2000). The carrying capacity of water hyacinth was assumed to be 70 kg/m^2, as this was approximately the maximum observed in the field (Reddy and D'Angelo, 1990).

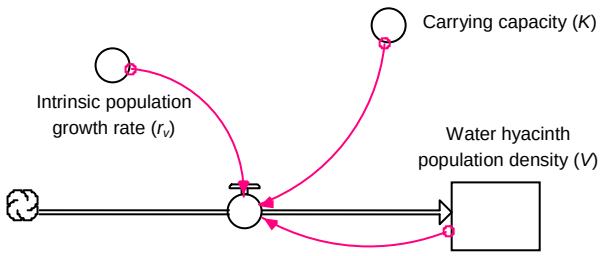


Figure 3.2: Stage 1 (excluding temperature) model of logistic water hyacinth growth in STELLA. The water hyacinth population density (V) is modified by the intrinsic population growth rate (r_v) (0.052 g/g/day; Center *et al.*, 1982) and the system carrying capacity (K) (70 kg/m^2; Reddy and D'Angelo, 1990).

Biomass density has been selected as the state variable (a variable which describes the system at any moment in time) as it reflects the bulk of the weed, and hence the scale of the water hyacinth problem (Wilson *et al.*, 2001). The initial population in each model was set to the mean biomass density for that particular site in the first month of sampling by Byrne *et al.* (2010). Fresh weights of water hyacinth biomass were used throughout this study in order to be comparable with values measured in the field (Byrne *et al.*, 2010).

The effect of temperature on water hyacinth populations

The Stage 1 (excluding temperature) water hyacinth growth model was developed into the Stage 2 (temperature) water hyacinth model (Figure 3.3). New model elements are shown in orange while any pre-existing model elements that have been altered are crosshatched.

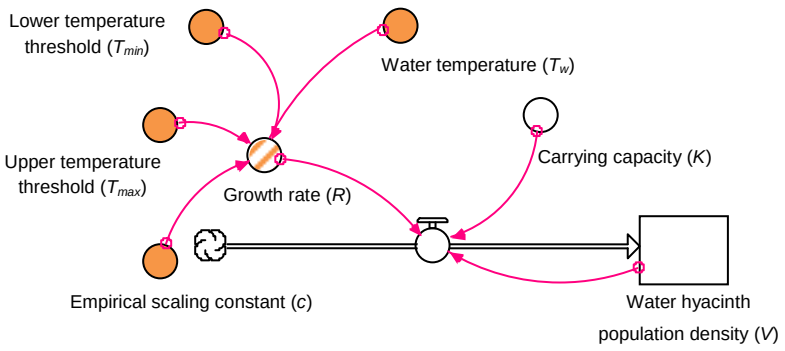


Figure 3.3: Stage 2 (temperature) model of logistic water hyacinth population growth in STELLA. The relative growth rate of the population (R) is modified by variable water temperature (T_w) according to van der Heide *et al.*, (2006). New model elements are shown in solid orange while previous model elements that have been altered are crosshatched in orange.

The Stage 2 (temperature) models of water hyacinth included variable water temperature. This temperature effect was defined by the general relationship between temperature and growth rate of floating macrophytes (van der Heide *et al.*, 2006)

$$R(T) = cT(T - T_{min})(T_{max} - T)$$

(Equation 5)

where R is the relative growth rate of the macrophyte population (g/g/day) and T is the water temperature (°C). T_{max} is the maximum growth temperature threshold (°C) for water hyacinth, above which the growth rate will decline; T_{min} is the minimum growth temperature threshold (°C) of water hyacinth, below which water hyacinth growth will decline; and c is the empirical scaling constant. The minimum and maximum growth thresholds for water hyacinth were defined as 8°C and 40°C respectively (Wilson *et al.,* 2005) and the empirical scaling constant c was defined as 8.7×10^{-6}, using growth rates and temperatures from Wilson *et al.,* (2005). The Stage 2 (temperature) model was run for two sites, Mbozambo Swamp and Delta Park, using the daily means of hourly water temperature records from Byrne *et al.* (2010). All field data were collected at sites that were under biological control management. As a result, no data were available to compare model outputs to conditions of unimpeded hyacinth growth. All water hyacinth model variables and parameters can be found in Table A.1.

3.2.3 Modelling *Neochetina eichhorniae* populations

Overview

Temperature is a basic driver of insect development (Campbell *et al.,* 1974) and has been used to determine the duration of each life stage in all weevil models. Stage 1 (constant oviposition) *N. eichhorniae* models were initiated by building up model modules of egg, larva, pupa and adult life stages. Constant oviposition rates and 100% survival between life stages were assumed. This model was then developed into Stage 2 (temperature-dependent) models to include both the temperature-dependent stage-specific survival and temperature-driven oviposition by the weevil, and then Stage 3 (winter mort.) models that incorporated age-dependent oviposition and stage-dependent winter mortality. Although the methods of all three of the model stages have been included, only Stage 2 and 3 models will be discussed in Chapter 4.

The insect development parameters; the thermal constant (K) and lower developmental threshold (t) for each life stage of *N. eichhorniae*, were taken from

King (2011) who used the reduced major axis regression, proposed by Ikemoto and Takai (2000) on data drawn from the literature (Table 3.2; DeLoach and Cordo, 1976; Stark and Goyer, 1983; El Abjar and Bashir, 1984; Shih *et al.*, 1994, Chikwenhere, 2000; Wilson, 2002; Coetzee, unpub.). The sum of life stage thermal constants was used to calculate the degree-day requirements for the entire life cycle (egg to adult) and the mean *t* estimated for each life stage determined the developmental threshold of *N. eichhorniae*. Although *N. eichhorniae* egg development *K* and *t* were defined by King (2011; n=11), the results of the egg development and survival experiment (Section 2.3.1) from the present study were used in this model instead (n=101). Unfortunately, data used in the model for larval and pupal life stages was limited and contained only three replicates per life stage (Table 3.2).

Table 3.2: Lower developmental thresholds (*t*) and thermal constants (*K*) for immature stages of *Neochetina eichhorniae* using the reduced major axis regression. (Adapted from King, 2011)

Life Stage	n	t ± SE (°C)	K ± SE (°D)	Used in models?
Egg	11	15.2 ± 2.2	94.7 ± 22.2	No
Larva	3	5.2 ± 4.3	976.3 ± 184.9	Yes
Pupa*	3	6.7 ± 4.7	242.7 ± 89.3	Yes
All	-	9.0	1313.7	No

*Data substituted from *N. bruchi*

Stage 1 insect models

In the STELLA programme, population stocks can be defined in various ways, depending on how the population stock needs to function. Defining a stock as an "array" allows the model to be replicated and run simultaneously. As such, stocks and associated flows and converters have been arrayed, replicating the model for each day of the model cycle (730 days). This allows the model to capture events, such as the number of eggs laid on each day and follow those eggs through the entire model cycle. All variables and parameters for Stage 1 weevil models can be found in Table A.2 in the Appendix.

Egg model module

Initially, a simple egg model was created (Figure 3.4). The egg density (E_d) on each day is directly influenced by the number of new eggs laid as well as the number of eggs hatching on that day, such that

$$E_{d_{(t)}} = E_{d_{(t-dt)}} + (E_n - E_m) * dt$$

(Equation 6)

where E_n and E_m are the new eggs and eggs hatching on each day respectively and *dt* is Delta time. Each of the egg density (E_d) arrayed stocks represents the current eggs/m^2 from a particular day. Therefore, the total egg density of the system (E) is determined by

$$E_{(t)} = Arraysum(E_d[*])$$

(Equation 7)

where the function *Arraysum* allows all elements [*] of the array (*i.e.* all 730 days) to be summed to give the total egg density at any given time.

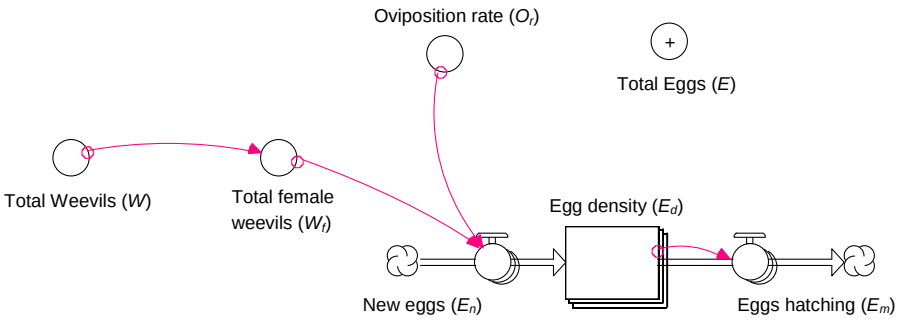


Figure 3.4: Simple egg model. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with + function as sums of allocated arrays (egg density).

The number of new individuals entering the egg life stage at any time is dependent on the rate of oviposition as well as by the number of females in the population and was defined as follows:

$$E_{n(t)} = O_{r(t)} * W_{f(t)}$$

(Equation 8)

Where O_r is the oviposition rate and W_f is the number of female weevils present in the population at that point in time. For determining the oviposition input by female weevils, the sex ratio of the population has been assumed at 1♀:1♂. The oviposition rate, O_r, was set at a value of 0.75 eggs/weevil/day for Delta Park (14°C) and 4 eggs/weevil/day for Mbozambo Swamp (24.5°C) using the relationship between oviposition and temperature shown by DeLoach and Cordo (1976).

Insect development time is vital in determining the population density in each life stage at a particular point in time and determining the available degree-days for insect development is the key to determining accurate developmental times. As such, before the number of eggs hatching per day (E_m) could be determined, a simple degree-day model was constructed (Figure 3.5) to track the cumulative degree-days that individual eggs would be exposed to during their development.

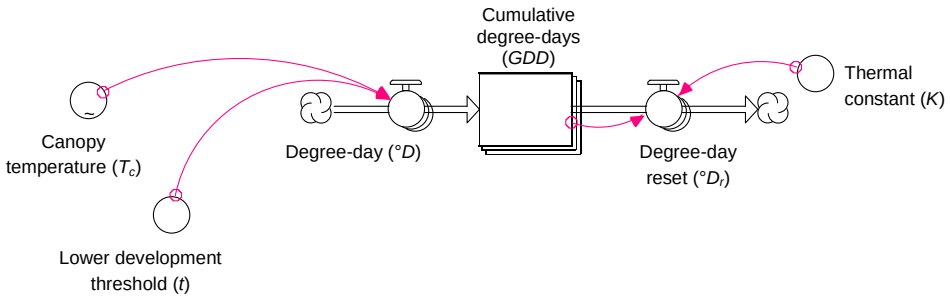


Figure 3.5: Simple degree-day model. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph.

Cumulative or gross degree-days (*GDD*) were determined by the available degree days (°*D*) and the degree-day reset (°D_r) functions such that

$$GDD_{(t)} = GDD_{(t-dt)} + (°D - °D_r) * dt$$

(Equation 9)

The available degree-days (°*D*) were calculated using

$$^{\circ}D_{(t)} = T_c - t$$

(Equation 10)

where T_c is daily mean canopy temperature and *t* is the lower developmental threshold. Canopy temperature is the microclimate temperature among the water hyacinth leaves. This source of temperature data was used as it affects weevil populations more directly because both eggs and weevils are predominately found in the water hyacinth canopy (King, 2011). The variable temperatures used in the insect models were the daily means of hourly canopy temperature records from Byrne *et al.* (2010).

Degree-days are not accumulated endlessly but rather accumulate up to a threshold, which normally results in an insect moving from one life stage to another. To allow for this, the cumulative degree-day per daily cohort was reset through the degree-day reset function (°D_r), described by

$$^{\circ}D_{r(t)} = if\ GDD_{(t)} \geq K\ then \frac{GDD_{(t)}}{dt}\ else\ 0$$

(Equation 11)

Where *GDD* is the cumulative degree-day and *K* is the thermal constant.

This simple degree-day model was then integrated with the simple egg model to form the Stage 1 (constant oviposition) egg model module (Figure 3.6). By integrating the models, the egg developmental period could be determined. The number of eggs hatching at any time (E_m) was thus defined as

$$E_{m(t)} = if\ GDD_{E(t)} \geq K_E\ then \frac{E_{d(t)}}{dt}\ else\ 0$$

(Equation 12)

Where GDD_E is the cumulative egg degree-days, K_E is the specific egg thermal constant, and E_d is the egg density at any time.

As part of this integration process, two new converters were added. The first converter was the *Day counter*, which tracked the days passed from the start of the model run. The second was the *Initiator* (*i*) which acted as a "green flag" for various processes to begin. The *initiator* converter was arrayed and was defined as

$$i[*] = if\ Day\ counter = [*]\ then \frac{Day\ counter}{dt}\ else\ 0$$

(Equation 13)

Where [*] indicates the specific array element or specific day model. This means that the initiator for a specific day (e.g. *i*[23]) would only hold a value other than zero on that particular day (e.g. day 23).

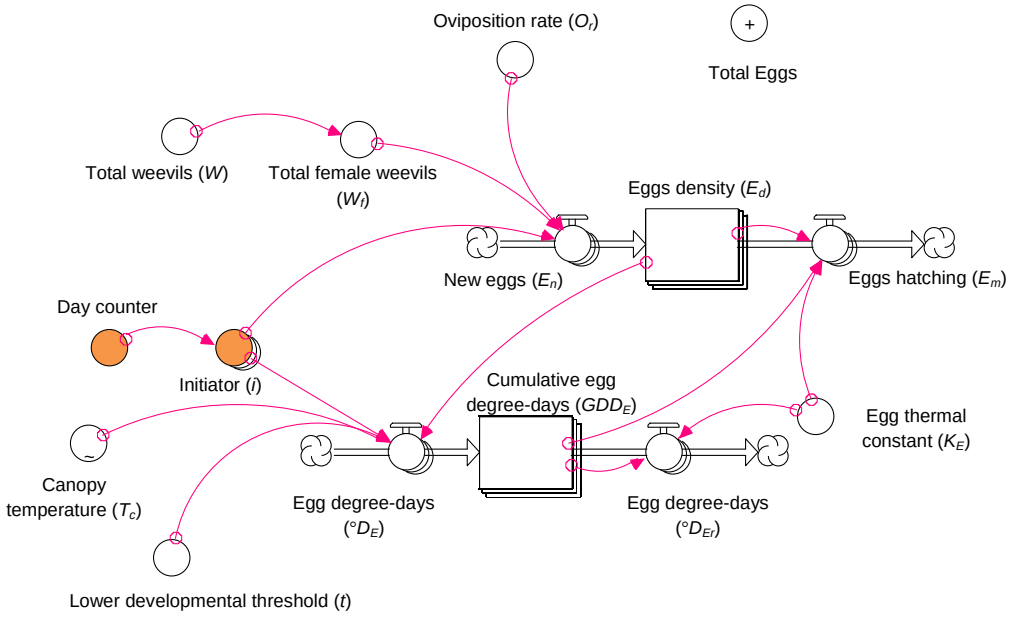


Figure 3.6: Stage 1 (constant oviposition) egg life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (egg density). New model elements are shown in solid orange.

By introducing the *initiator*, the formulae for new eggs (E_n) changed as follows

$$E_n[*] = if\ i[*] \geq 0\ then\ (O_{r_{(t)}} * W_{f_{(t)}})\ else\ 0$$

(Equation 14)

Where [*] indicates the specific array element or specific day model. Likewise, the available degree-days ($°D$) was changed to

$$°D[*] = if\ i[*] \geq 0\ and\ (T_c - t) \geq 0\ and\ E_d[*] > 0$$
$$then\ (T_c - t)\ else\ 0$$

(Equation 15)

If the conditions (Equation 14) were met then the model allowed degree-days to be available and accumulated for use in the model.

Larval and pupal model modules

Once the egg module was created, a larval (Figure 3.7) and then a pupal (Figure 3.8) module was built.

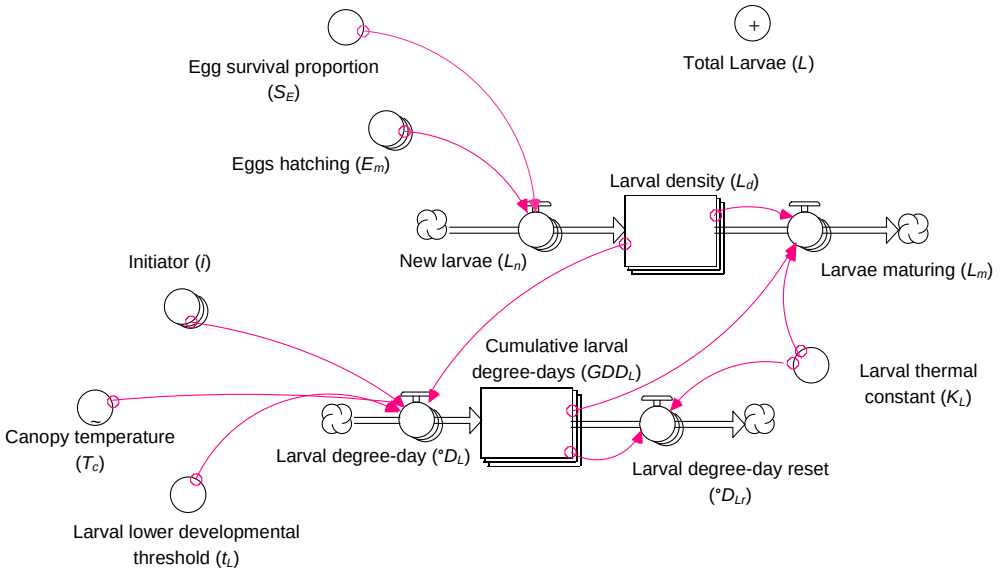


Figure 3.7: Stage 1 (constant oviposition) larval life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (larval density).

Similar to the egg module, the larval (L_d) and pupal (P_d) densities from any given day are directly influenced by the number of new individuals entering the stage and old individuals maturing and leaving the stage. These relationships are described by

$$L_{d(t)} = L_{d(t-dt)} + (L_n - L_m) * dt$$

(Equation 16)

$$P_{d(t)} = P_{d(t-dt)} + (P_n - P_m) * dt$$

(Equation 17)

where L_n and P_n are the new larvae and pupae entering their respective life stages and L_m and P_m are those individuals maturing from the larval and pupal stages respectively. As with the egg model, each of the larval density (L_d) and pupal density (P_d) arrayed stocks represents the current larvae or pupae/m^2 from a particular day. Therefore, the total larval (L) and pupal (P) densities of the system are determined by the sum of arrays,

$$L_{(t)} = Arraysum(L_d[*])$$

(Equation 18)

$$P_{(t)} = Arraysum(P_d[*])$$

(Equation 19)

Where [*] indicates the specific array element or specific day model. New larvae and pupae entering the system are a function of the how many eggs survive to hatch and how many larvae survive to pupate. In this model, it is assumed that all eggs are available to hatch but only a fraction of those will survive to become larvae, while all larvae are available to pupate but only a fraction will survive to become pupae. The number of new larvae (L_n) and new pupae (P_n) is thus determined by

$$L_n[*] = E_m[*] * S_e$$

(Equation 20)

$$P_n[*] = L_m[*] * S_L$$

(Equation 21)

respectively, where E_m and L_m are the numbers of eggs and larvae available to hatch and pupate respectively and S_e and S_L are the respective egg and larval survival proportions. In stage 1 models, all survival was kept at 100%.

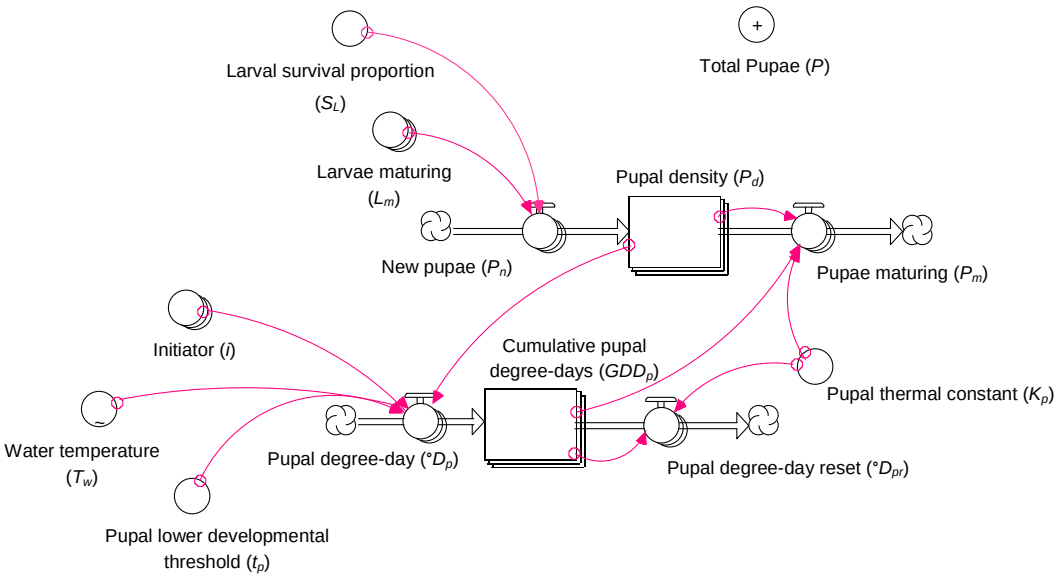


Figure 3.8: Stage 1 (constant oviposition) pupal life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (pupal density).

Although the model structures for calculating degree-days is the same for all model modules (Equation 9; 10; 11; 15), substitution of the relevant egg, larval or pupal variables occurs, allowing degree-days to accumulate according to the specific life stage involved. An important substitution occurs in the calculations of pupal degree-days. Instead of using canopy temperature (T_c), water temperature (T_w) is used as pupae form balls on the submerged roots of water hyacinth plants (DeLoach and Cordo, 1976; Visalakshy and Jayanth, 1996). These specific larval (GDD_L) and pupal (GDD_P) degree-day accumulations determine when larvae and pupae mature in the following ways, allowing individuals to move into the next life stage.

$$L_{m_{(t)}} = if\ GDD_{L_{(t)}} \geq K_L\ then \frac{L_{d_{(t)}}}{dt}\ else\ 0$$

(Equation 22)

$$P_{m_{(t)}} = if\ GDD_{P_{(t)}} \geq K_P\ then \frac{P_{d_{(t)}}}{dt}\ else\ 0$$

(Equation 23)

Adult model module

The adult model module was constructed using a different type of stock (Figure 3.9).

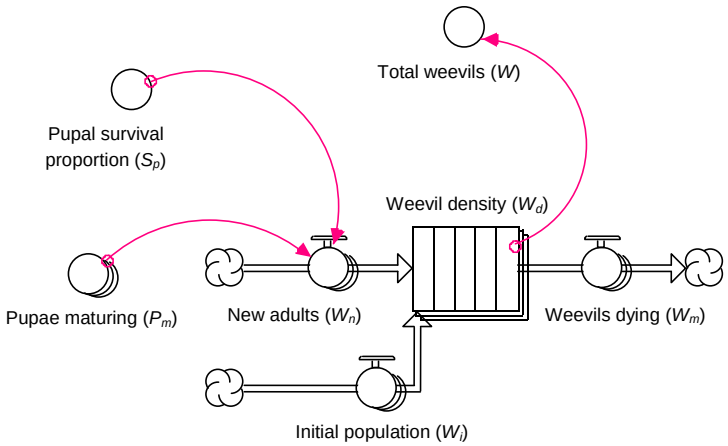


Figure 3.9: Stage 1 (constant oviposition) adult life stage model module. Stage 1 models assume 100% survival between life stages and constant oviposition rates based on mean site temperatures. Layered stocks, flows and converters are indicative of variables that have been arrayed.

In all the immature life stages, density stocks were defined as arrayed reservoirs, which were influenced by accumulating degree-days. The adult stage in this model, however, is not temperature-dependent and adults have been assumed to have a constant longevity of 104 days (Jianqing *et al.*, 2002). Because of the fundamental difference in the modelling, the type of stock used for adult populations was arrayed "conveyors". Conveyer stocks work differently to reservoir stocks in that instead of accumulating individuals through in- and out-flows, individuals are deposited onto the conveyor and carried for a specified period before being unloaded. This allows adult weevils to be deposited into the

system, and remain in the system for only as long as their specified longevity, which is *not* temperature-dependent.

An additional term of *initial population* (W_i) has been included in weevil density (W_d) calculations to represent agents being released at a new site, such that

$$W_{d_{(t)}} = W_{d_{(t-dt)}} + (W_n + W_i - W_m) * dt$$

(Equation 24)

where W_n and W_m are new weevil adults and dying adults respectively.

It should be noted that a maximum population density of 150 weevils/m^2 was set, as a conservative estimate based on maximum densities observed in the field (Center and Durden, 1986). In order for this carrying capacity to take effect, the following calculation for total weevils (W) was used

$$W_{(t)} = if\ Arraysum(W_d[*]) \geq 150\ then$$

$$Arraysum(W_d[*])\ else\ if\ Arraysum(W_d[*]) > 150\ then\ 150\ else\ 0$$

(Equation 25)

Where W_d[*] is the weevil density for a specific array element or specific day model. The initial population (W_i) was set to 100 weevils/m^2, and was defined by

$$W_{i_{(t)}} = if\ time \leq 0\ then\ 100\ else\ 0$$

(Equation 26)

so that 100 weevils/m^2 would only be added once, at model initiation, to represent weevils being released at a site.

All model modules above formed the entirety of the Stage 1 (constant oviposition) weevil model that includes constant oviposition (based on mean site temperatures) and 100% survival between life stages of the insect.

Stage 2 insect models

Stage 2 (temperature-dependent) insect models were developed by introducing temperature-dependent oviposition (Figure 3.10) and temperature-dependent egg survival (Figure 3.11).

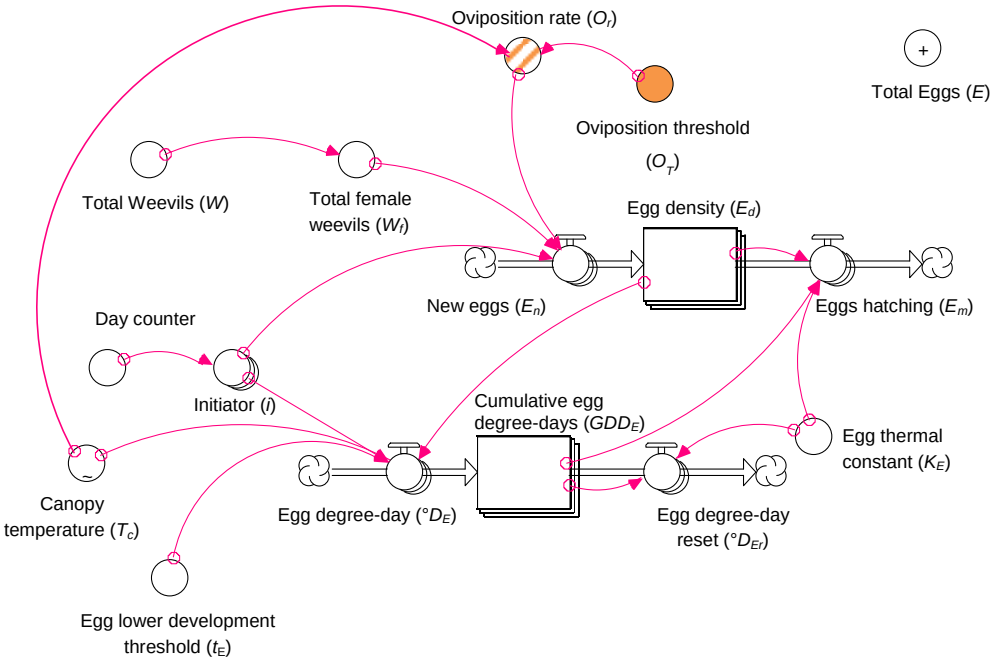


Figure 3.10: Stage 2 (temperature-dependent) egg life stage model module. Stage 2 models assume temperature-dependent oviposition rates (O_r; DeLoach and Cordo, 1976). Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (egg density). Variables that have been altered from Stage 1 models are crosshatched in orange, while new elements are solid orange.

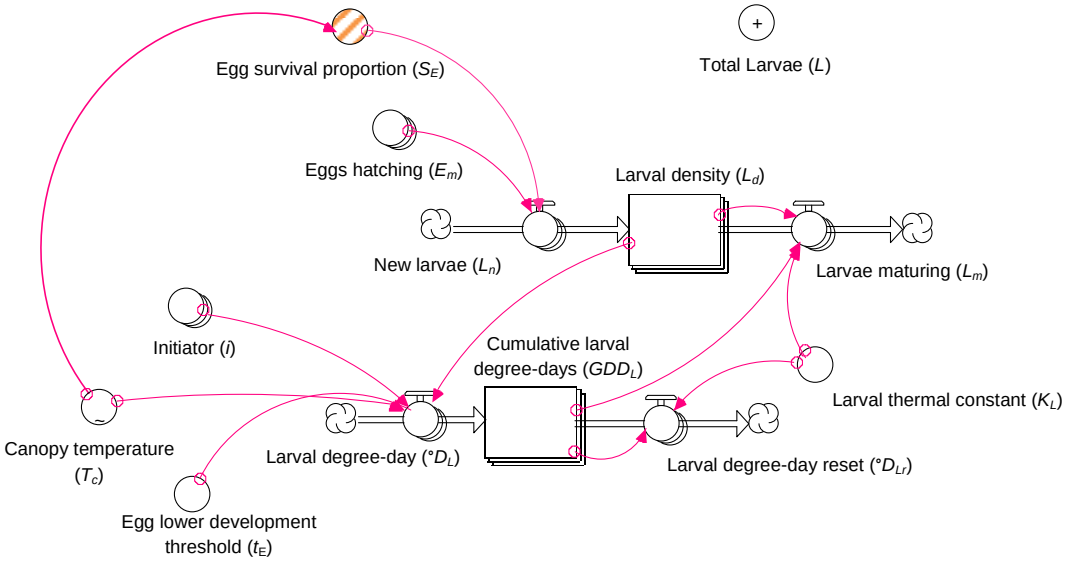


Figure 3.11: Stage 2 (temperature-dependent) larval life stage model module. Stage 2 models assume temperature-dependent egg survival proportions (S_E). Layered stocks, flows and converters are indicative of variables that have been arrayed. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (larval density). Variables that have been altered from Stage 1 models are crosshatched in orange.

Neochetina *oviposition*

A third order polynomial regression was used to determine the mathematical relationship between oviposition and temperature in Microsoft Excel using data drawn from DeLoach and Cordo (1976; Table 3.3).

Table 3.3: Oviposition rate by *Neochetina eichhorniae* at six constant temperatures (Taken from DeLoach and Cordo, 1976).

Temperature (°C)	**Oviposition Rate** (egg/female/day)
10	0.30
15	0.75
20	2.10
25	3.90
30	7.00
35	5.50

The resulting formula (Equation 27) was used to describe the temperature-dependent oviposition rate (O_r) in the Stage 2 (temperature-dependent) insect models. A temperature threshold for oviposition was assumed at 10°C due to the low oviposition rates recorded (Table 3.3). For use in Stage 2 (temperature-dependent) models oviposition rates were defined such that O_r remains positive above the assumed oviposition temperature threshold (O_T) of 10°C,

$$O_r = \ if\ (T_c \geq O_T)\ then$$

$$(-0.0018 + 0.1228T_c^2 - 2.2192T_c + 12.224\ )\ else\ 0$$

(Equation 27)

Additional variables and parameters found in Stage 2 insect models can be found in Table A.3 in the Appendix.

Insect survival

A constant stage-specific survival proportion *S* was determined for each life stage. This proportion was used to determine how many individuals from a preceding life stage would survive to enter the following life stage. The larval (S_L) and pupal (S_P) life-stage survival proportions were set at 0.85 and 0.95 respectively (DeLoach and Cordo, 1976). The egg survival proportion (S_E), however, was made temperature-dependent and was determined from empirical data from the egg survival proportion (S_E) results of the egg development and survival experiment (Figure 2.2). However, in order for egg survival proportion to remain a positive fraction the below model calculation was used

$$S_E = if\ (-0.0077T_c^2 + 0.3754T_c - 3.7266) \geq 0\ then$$

$$(-0.0077T_c^2 + 0.3754T_c - 3.7266)\ else\ 0$$

(Equation 28)

Stage 3 insect models

Stage 3 (winter mortality) insect models were developed by incorporating egg and larval stage-dependent winter mortality (Figure 3.12) and age-dependent oviposition (Figure 3.13).

Winter mortality

Winter mortality was introduced by incorporating daily minimum temperatures (Figure 3.12). It was assumed that each low temperature event (canopy temperatures below 0°C) would result in a loss of 10% of the egg population and 10% of the early instar larval population (larvae that had accumulated less than two thirds of the larval thermal constant). These "winter mortality" losses were defined by

$$E_{w(t)} = if\ T_{c_{min}} \leq 0\ then\ \left(0.1 * \frac{E_{d(t)}}{dt}\right) else\ 0$$

(Equation 29)

$$L_{w(t)} = if\ T_{c_{min}} \leq 0\ and\ GDD_L < (0.6 * K_L) then\ \left(0.1 * \frac{L_{d(t)}}{dt}\right) else\ 0$$

(Equation 30)

where E_w and L_w are the winter mortality losses for the egg and larval populations, E_d and L_d are the egg and larval population densities respectively and GDD_L and K_L are the larval cumulative degree-days and thermal constant respectively.

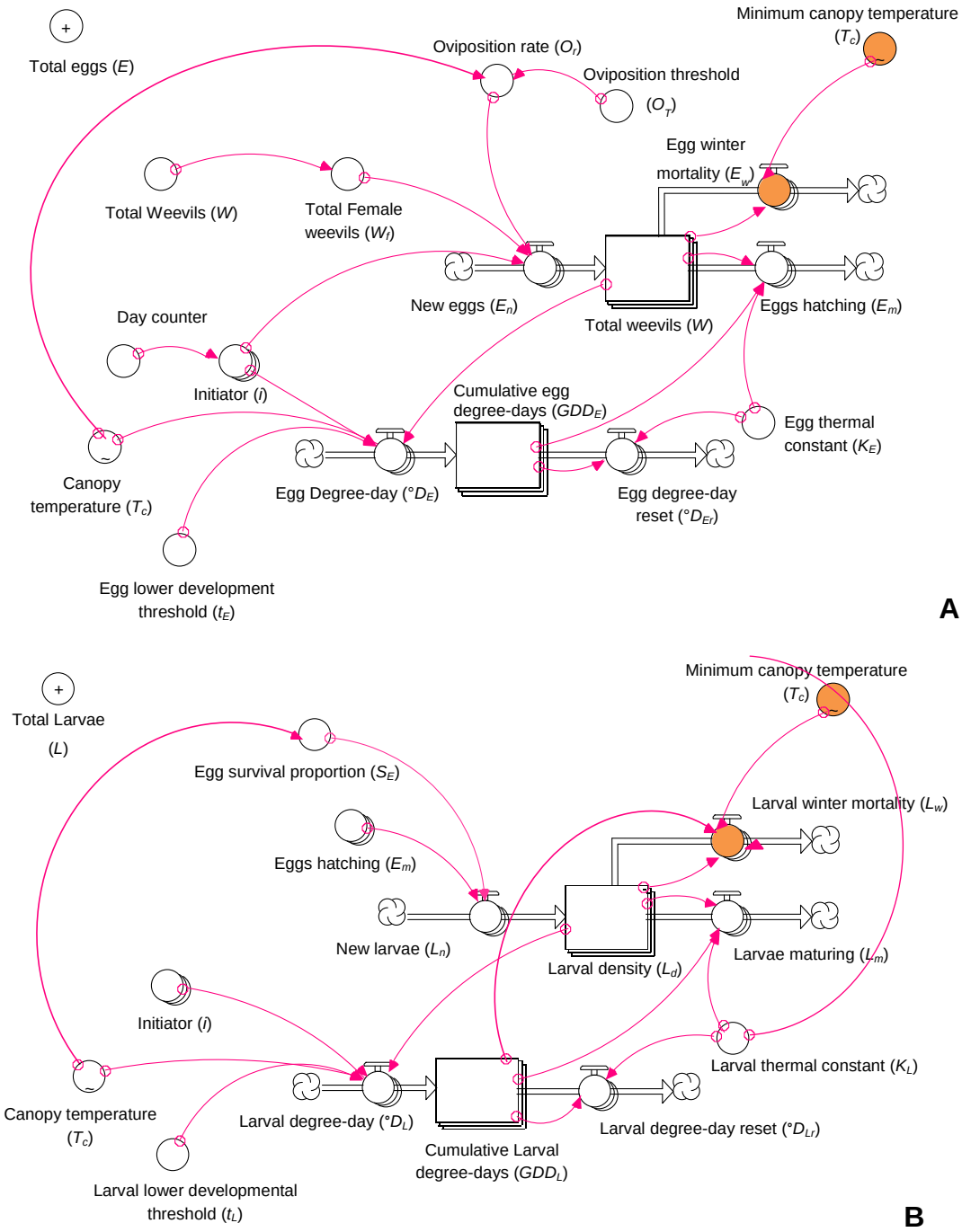


Figure 3.12: Stage 3 (winter mortality) A. egg and B. larval life stage model modules. Stage 3 models assume temperature-dependent oviposition rates (O_r; DeLoach and Cordo, 1976) and winter mortality. Layered stocks, flows and converters are arrayed variables. Converters marked with ~ can vary over time and retrieve data from a temperature graph. Converters marked with + function as sums of allocated arrays (A. egg density; B. larval density). New variables that have been introduced to Stage 2 models are solid orange.

Age-dependent oviposition

Neochetina weevils oviposit approximately 95% of their egg contribution with in the first 33 days, 50% of which are oviposited in the first 7 days after eclosion (DeLoach and Cordo, 1976; Akbay *et al.*, 1991). As such, age-structure was incorporated into the adult module of Stage 3 models (Figure 3.13).

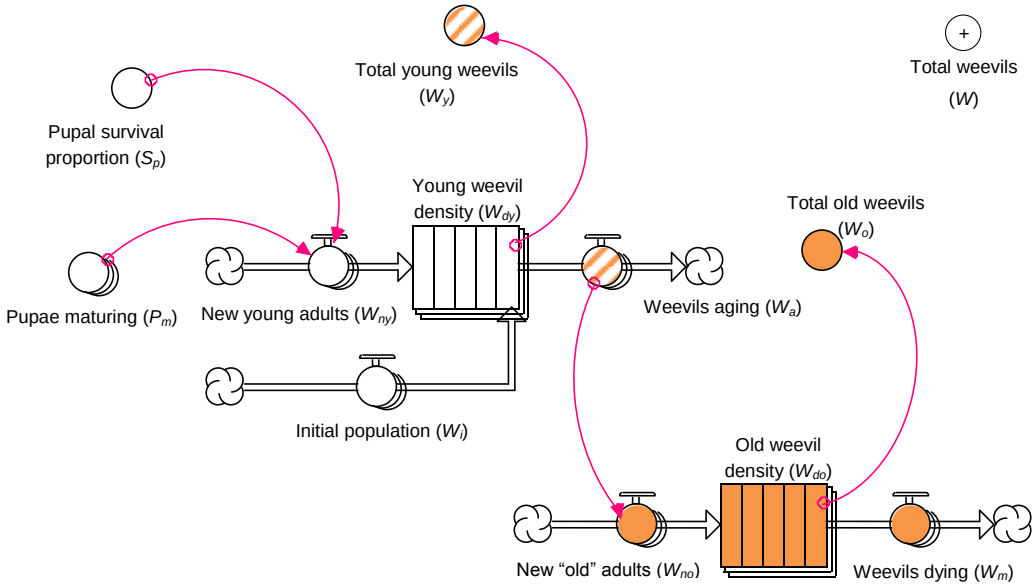


Figure 3.13: Stage 3 (winter mortality) adult life stage model module. Stage 3 models assume that adult weevils oviposit at maximum rates for only 21 days of the 104-day life span. Layered stocks, flows and converters are arrayed variables. Converters marked with + function as sums of allocated arrays (total old + young weevils). New variables that have been introduced to Stage 2 models are solid orange.

Young weevils were defined as weevils that contributed to oviposition while old weevils did not oviposit. Young weevils were assumed to oviposit at maximum rates for the first 21 days after eclosion. After 21 days, weevils were transferred to the "old" weevil category through the *new "old" adults* inflow, defined by

$$W_{no_{(t)}} = W_{a_{(t)}}$$

(Equation 31)

where W_a is the weevil-aging period of 21 days. Weevils remained on the "old" conveyor for 83 days, giving adult weevils a total longevity of 104 days, as in previous models. Densities of young and old weevils were determined (Equation

24) by substituting W_d (weevil density) with W_{dy} and W_{do} and W_n (new weevils) with W_{ny} and W_{no} for young and old weevils respectively. Total weevils (W) was determined by,

$$W_{(t)} = W_{y_{(t)}} + W_{o_{(t)}}$$

(Equation 32)

where W_y and W_o are determined using (Equation 25). Splitting the adult population into two age categories resulted in a doubling of the weevil carrying capacity to 300 adults/m^2. All other adult module variables were defined as in Stage 2 models. Additional variables and parameters found in Stage 3 insect models can be found in Table A.4 in the Appendix.

3.2.4 Combined water hyacinth biocontrol models

Neochetina eichhorniae *feeding*

Before water hyacinth and weevil models could be integrated, weevil-feeding relationships were determined. In this study, temperature-dependent feeding by adult and larval *N. eichhorniae* was explored.

Adult feeding

Neochetina eichhorniae feed on water hyacinth leaves making regularly shaped abrasions or scars on the lamina surface (DeLoach and Cordo, 1976; Franceschini *et al.*, 2010). It is also widely known that weevil herbivory differs with temperature (Shih *et al.*, 1994; Chikwenhere, 2000; Jianhao *et al.*, 2003). In order to determine a temperature-dependent adult feeding relationship, a third order polynomial regression was used to determine the mathematical relationship in Microsoft Excel using data from the literature (Table 3.4) combined with results from medium nutrient feeding results from this study (Section 2.3.3). All data recorded as feeding scars were converted to leaf area removed (mm^2/weevil/day) by using the conversion factor of 4.5mm^2/scar (DeLoach and Cordo, 1976).

Table 3.4: Leaf area removed from water hyacinth leaves through adult *Neochetina eichhorniae* herbivory

Temperature (°C)	**Leaf area removed (mm^2/weevil/day)**		
Source	*1*	*2*	*3*
5	-	-	0.03
10	10.00	-	-
12	-	-	8.06
15	24.30	13.95	22.95
20	44.30	65.25	37.89
25	63.60	62.28	-
30	86.30	111.65	-
35	85.00	95.36	-

1. DeLoach and Cordo (1976); 2. Shih *et al.*, (1994); 3. King (2011)

Larval feeding

The larval feeding rate (0.9 g/larva/day) was determined for 25°C in Section 2.3.2. It was assumed that temperature-dependent larval feeding would follow the same pattern as adult temperature-dependent feeding. A conversion factor was determined for 25°C using the following equation,

$$Conversion\ factor\ (L_f) = \frac{estimated\ adult\ feeding\ rate}{empirical\ larval\ feeding\ rate}$$

(Equation 33)

where the estimated adult feeding rate is the regression value at 25°C and the empirical larval feeding rate is 0.9 g/larva/day. Once this conversion factor was determined, the larval feeding rates at various temperatures were calculated and regressed to provide a larval feeding equation.

Plant and insect model integration

Stage 4 (integrated) water hyacinth and insect models were developed by integrating Stage 3 (winter mortality) insect models with Stage 2 (temperature) water hyacinth models. This was achieved by introducing herbivory removal to the water hyacinth models (Figure 3.14). Stage 4 models were built up in multiple phases, introducing constant then temperature-dependent adult and larval

herbivory and then a larval carrying capacity. However, only methods describing Stage 4 (integrated) (Figure 3.14) and Stage 4*b* (larval carrying capacity) models have been included.

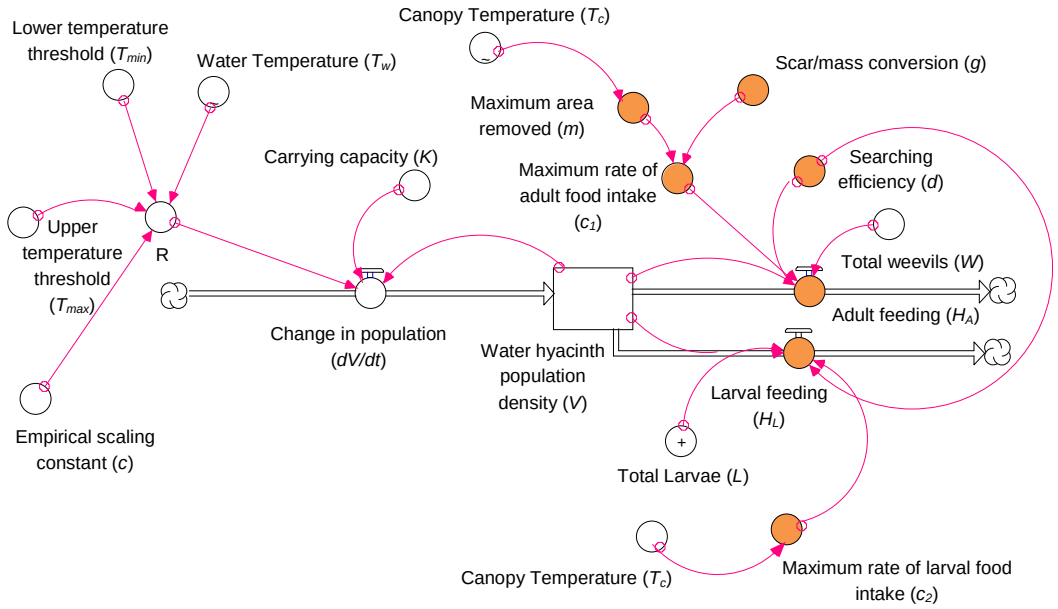


Figure 3.14: Stage 4 (integrated) model. Stage 4 models assume variable water and canopy temperatures (T_w, T_c), maximum area removed through adult herbivory (m) as well as variable maximum rate of larval herbivory (c_2). Converters marked with ~ can vary over time and retrieve data from a temperature graph. New model elements are indicated in solid orange.

Stage 4 (integrated) model

Water hyacinth models were described as in Section 3.2.2 (Figure 3.3) with additional terms for adult herbivory (H_A) and larval herbivory (H_L), such that the water hyacinth population density (V) was described as follows (Figure 3.14),

$$V_{(t)} = V_{(t-dt)} + \left(\frac{dV}{dt} - H_A - H_L\right) * dt$$

(Equation 34)

where *dV/dt* is the change in the water hyacinth population. Adult herbivory (H_A) and larval herbivory (H_L) were determined using the herbivory term from Caughley and Lawton's (1981) "*Laissez-faire"* model,

$$H_{A_{(t)}} = c_1 * W_{(t)} * \left(1 - EXP(-d * V_{(t)}\right)$$

(Equation 35)

$$H_{L_{(t)}} = c_2 * L_{(t)} * \left(1 - EXP(-d * V_{(t)}\right)$$

(Equation 36)

where c_1 (kg/weevil/day) and c_2 (kg/larva/day) are the maximum rate of food intake per weevil and larva respectively. *W* and *L* are the total weevil and larval densities, *d* is the searching efficiency and *V* is the water hyacinth density. The searching efficiency of the herbivore (*d*) was assumed to be one, as *Neochetina* weevils live on the vegetation source they require as food, do not consume the entire plant, and hence will spend no time searching for food. The maximum rate of food intake per weevil was defined as,

$$c_{1_{(t)}} = m * g$$

(Equation 37)

where *m* is the maximum area removed per weevil (determined from literature and experiments) and *g* is the scar/mass conversion factor of $1.73*10^{-7}$ kg/mm^2 determined from Franceschini (*pers. comm.*). The maximum area removed (*m*) was made temperature-dependent, using the temperature-dependent feeding relationship derived in the *Adult feeding* portion of Section 3.2.4 such that,

$$m_{(t)} = if\ (-0.0114 * T_c^3 + 0.6394 * T_c^2 - 5.4516 * T_c) > 86.3\ then\ 86.3\ else\ if$$

$$(-0.0114 * T_c^3 + 0.6394 * T_c^2 - 5.4516 * T_c) \geq 0\ then$$

$$(-0.0114 * T_c^3 + 0.6394 * T_c^2 - 5.4516 * T_c)\ \ else\ 0$$

(Equation 38)

where 86.3mm^2 is considered the maximum *area* that can be removed per weevil per day (DeLoach and Cordo, 1976). DeLoach and Cordo (1976) were one of the few authors to measure precise area removed, which is why their maximum

feeding rate has been used. The maximum *biomass* removal possible is therefore 1.49 x 10^{-5} kg/weevil/day. However, weevil biomass removal and the weevil-borne microbes introduced by weevil feeding both influence water hyacinth growth (18% and 19% respectively; Venter *et al.,* 2013). The maximum rate of food intake (c_1) was therefore doubled for Stage 4 models to account for the potential effects of weevil-borne microbes on water hyacinth growth.

The maximum rate of food intake per larva c_2 was temperature-dependent and defined as,

$$c_{2(t)} = if\ (-0.0001 * T_c^3 + 0.0038 * T_c^2 - 0.0576 * T_c - 2 * 10^{-14}) \geq 0\ then$$

$$(-0.0001 * T_c^3 + 0.0038 * T_c^2 - 0.0576 * T_c - 2 * 10^{-14})\ else\ if$$

$$(-0.0001 * T_c^3 + 0.0038 * T_c^2 - 0.0576 * T_c - 2 * 10^{-14}) > 1.1$$

$$then\ 1.1\ else\ 0$$

(Equation 39)

where 1.1g is considered the maximum biomass that can be removed per larva per day, and 0.9 g/larva/day is the feeding rate at 25°C. To convert from grams to kilograms c_2 was divided by 1000. All weevil egg, larval, pupal and adult modules remain as described in Stage 3 insect models.

Stage 4b (larval carrying capacity) model

In Stage *4b* a carrying capacity of 600 larvae/m^2 was introduced to the larval populations, such that the total larval population (L) was defined by,

$$L_{(t)} = if\ Arraysum(L_d[*]) \leq 600\ then\ Arraysum(L_d[*])\ else$$

$$if\ Arraysum(L_d[*]) > 600\ then\ 600\ else\ 0$$

(Equation 40)

Where L_d[*]indicates the specific array element or specific day model. The carrying capacity was estimated using the mean plant density and mean number of petioles per plant over 14 sites in South Africa (Byrne *et al.,* 2010). All other

model modules and elements remain as defined in Stage 4 (integrated) models. All models were run for the two sites Delta Park and Mbozambo Swamp.

4 CHAPTER FOUR – SIMULATION AND VALIDATION OF THE MODEL OF WATER HYACINTH BIOLOGICAL CONTROL

4.1 Introduction

Models are commonly used to study weed populations (Holst *et al.,* 2007), and are useful to weed management. Particularly with biological control systems, being able to simulate the system to select control agents could result in extensive savings, in both time and money (McEvoy and Coombs, 1999). By modelling or simulating biological control systems, researchers are able to explore the possible risks and benefits of introducing an agent (Raghu *et al.,* 2007; Mills and Kean, 2010), predict efficacy of biocontrol agents prior to release in the field and evaluate agents post-release (Sims *et al.,* 2006; Mills and Kean, 2010), as well as explore the interacting variables within the biological control system (Kriticos, 2003) in a fraction of the time it might take to do so in the field.

However, using valid models is also important when simulating these systems. Validation is therefore an important step in accepting models for use, particularly in the management of ecological systems. Decision makers require that models be 'validated' in some way, showing that they are sufficient representations of the real-world systems that they are trying to simplify. The term 'validation' though, has been under some scrutiny because of conflicting definitions and usage in modelling literature (Rykiel, 1996). Essentially, though, validation is the process of determining if the model is acceptable for its intended use and if confidence can be placed in the inferences from model results (Rykiel, 1996; Bennett *et al.,* 2013; Augusiak *et al.,* 2014).

Several methods of validation can be used under different criteria. Power (1993) used the criteria of *replicative, predictive* and *structural validity*, if models matched acquired data from the system (used in model construction), matched

independent data (not used in model construction), and if it reproduced real-world behaviour of the system, respectively. Rykiel (1996), however, used the criteria *operational, conceptual* and *data validity*, all three of which need to be met in order to validate a model. *Operational validity* is defined as the demonstration that model outputs meet the performance standard required for the model purpose, *conceptual validity* is the correctness of the underlying theories and assumptions of the model, and *data validity* is the assurance that data meet some specified standard and represent the real system as accurately as possible. More recently, five and six-step methods of validation have been proposed, combining the above-mentioned criteria with some additional techniques (Bennett *et al.*, 2013; Augusiak *et al.*, 2014).

Techniques of model validation are numerous and include subjective assessment, visual analysis, statistical analysis, and sensitivity analysis (Mayer and Butler, 1993; Power, 1993; Augusiak *et al.*, 2014; Bennett *et al.*, 2013), but the types of validation tests that can be conducted are limited by the available data and understanding of the system being modelled (Rykiel, 1996). Subjective assessments, such as Turing tests, can be used but by their nature are susceptible to bias (Law and Kelton, 1982; Mayer and Butler, 1993; Rykiel, 1996). Although time series plots of modelled and observed data are informative visual representations, observed vs. predicted data plots are preferred (Mayer and Butler, 1993). However, these observed vs. predicted data plots are considered insufficient (Augusiak *et al.*, 2014) and do not include the relationship of modelled variables with time (Bennett *et al.*, 2013), which is often important in ecological systems. Statistical analysis is a more robust comparison between modelled and observed data. A range of statistical tests exists (see McCarl, 1984) and is dependent on the data available. Model outputs that match observed data particularly well may result from the "fine-tuning" of several variables, which if changed may result in less ideal matches to observed data. A sensitivity analysis (the response of a model to changes in model inputs) is therefore important for identifying parameters that strongly affect model outputs (Augusiak *et al.*, 2014), and are often used as part of the model validation process.

Although all of these criteria and techniques exist for model validation, before validation can begin several questions need to be addressed (1) what is the purpose of the model; (2) What are the performance criteria of the model; (3) What is the (environmental) context of the model? (Rykiel, 1996). Here we consider that the population models of water hyacinth and *Neochetina eichhorniae* weevils that were developed in Chapter 3 (namely the final Stage 4*b* (larval carrying capacity) models) were developed to simulate water hyacinth biomass and *Neochetina* weevil populations over a two-year period. Models are expected to produce adequate estimations of both weevil and plant populations for the two-year simulation period, and have been built in the ecological context of changing temperature regimes and unlimited nutrients. These models will be validated against independent data (Byrne *et al.,* 2010) using time series plots (as seasonal changes in populations are important) and appropriate statistical analyses.

4.2 Methods

Model results are presented here, but only final Stage 4*b* (larval carrying capacity) models were validated. To determine the match between modelled and observed data outputs of Stage 4*b* (larval carrying capacity) models were compared to observed water hyacinth and weevil population data over a two-year period from Byrne *et al.,* (2010). Modelled populations were exported at a 30-day frequency to correspond with monthly data collections for each field site. Weevil adult numbers for field data were estimated from observed weevils/plant and plants/m^2, while larval populations were estimated from mined petioles/plant and plants/m^2. Modelled and observed field data were grouped into four seasons per year for statistical comparison, using a factorial ANOVA (data source (Model vs. Field) and season used as categorical predictors).

4.3 Results

4.3.1 Model simulation

Water hyacinth models

Logistic growth of water hyacinth

The STELLA Stage 1 (excluding temperature) water hyacinth model exhibited typical logistic growth behaviour for the weed (Figure 4.1). In the absence of abiotic and biotic pressures, at a constant intrinsic population growth rate (r_v=0.052 g/g/day) the water hyacinth biomass density reached the carrying capacity of 70 kg/m^2 within less than a year (111 days).

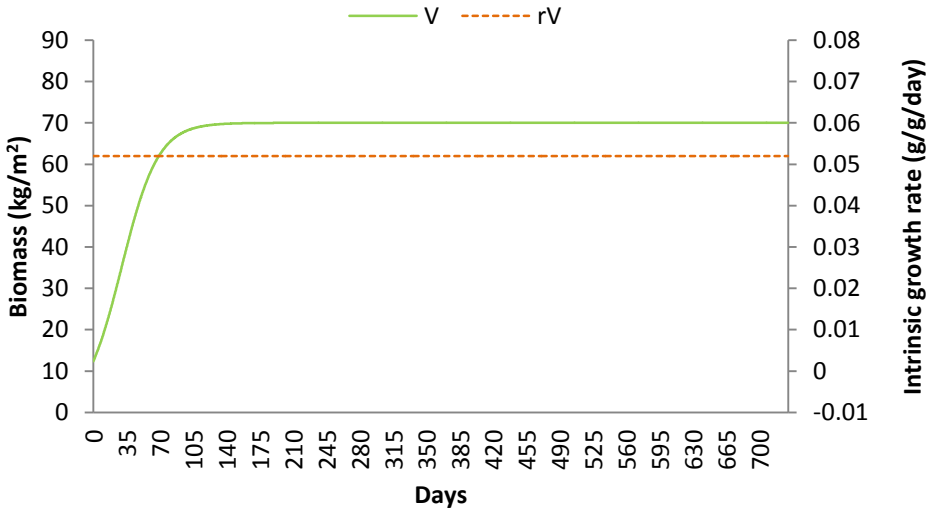


Figure 4.1: Stage 1 (excluding temperature) logistic growth model of water hyacinth; *V* is the water hyacinth biomass density and r_V is the intrinsic growth rate of water hyacinth 0.052 g/g/day; starting biomass 12.4 kg/m^2.

The effect of temperature on water hyacinth growth

Incorporating variable water temperature from the field approximated the seasonal temperature fluctuations and caused water hyacinth growth rates to vary between the cold and warm sites (Delta Park and Mbozambo Swamp respectively; Figure 4.2). Delta Park reached carrying capacity after 315 days while Mbozambo Swamp took only 92 days. The growth rate at Delta Park reached a maximum of 0.053 g/g/day and a minimum of -0.004 g/g/day, while the Mbozambo Swamp reached 0.058 g/g/day and 0.024 g/g/day respectively (Figure 4.2).

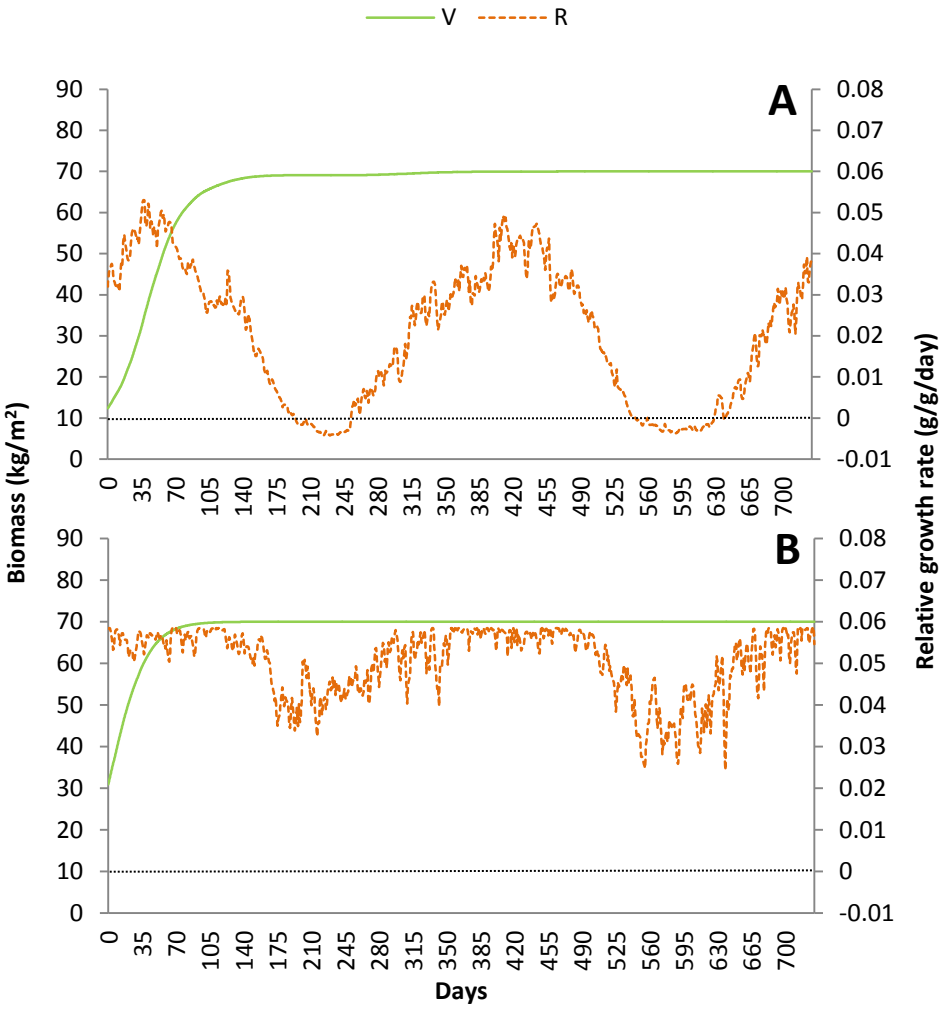


Figure 4.2: Output from Stage 2 (temperature) model of water hyacinth growth under A. low variable water temperatures (Delta Park: Monthly temperature minimum and maximum 5.4°C and 23.8°C respectively) and B. high variable temperatures (Mbozambo Swamp: monthly temperature minimum and maximum 15.4°C and 32.9°C respectively); *V* is the water hyacinth biomass density and *R* is the relative growth rate of water hyacinth under fluctuating temperatures. The dotted line indicates the point of zero population growth.

Neochetina eichhorniae models

Stage 2 (temperature-dependent) insect models

Both canopy and water temperature regimes differed considerably between sites, with Delta Park having, on average, 7.31°C cooler canopies and 13.95°C cooler waters than Mbozambo Swamp (Figure 4.3). Analysis of variance (ANOVA) of the daily minimum and maximum temperatures at each sites indicated a

significant difference in both canopy ($F_{1, 1312}$=2356.9; p<0.01) and water temperatures ($F_{1, 2918}$=3495.7; p<0.01).

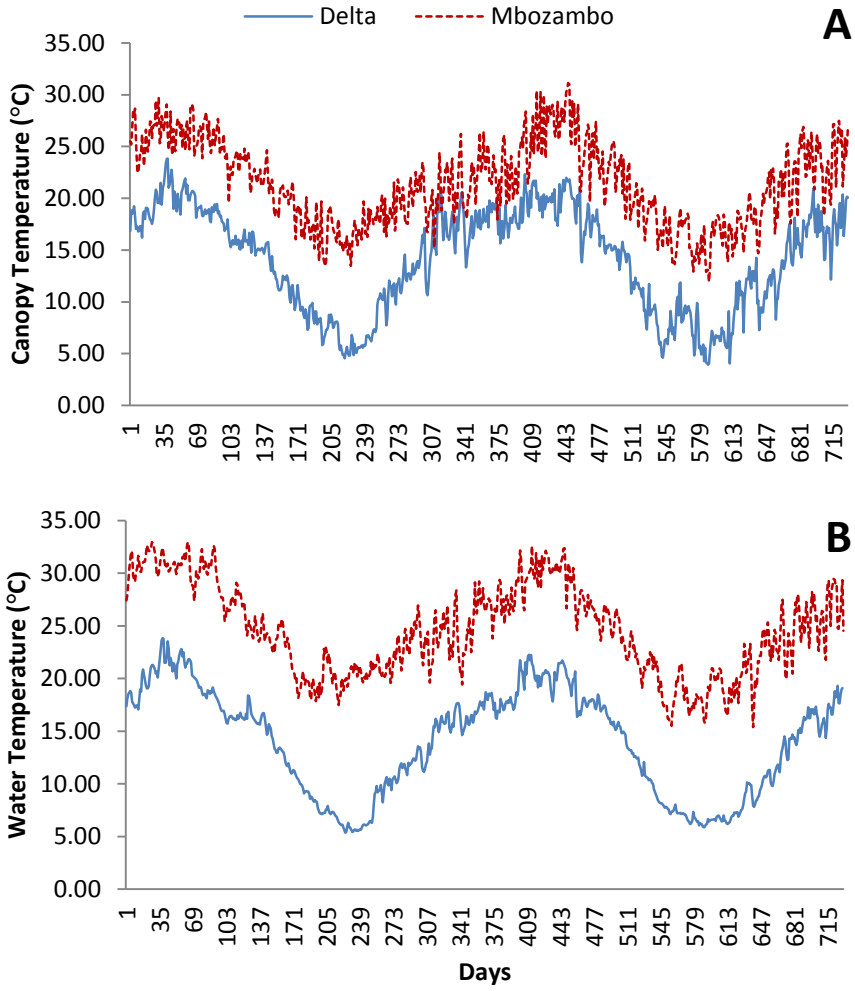


Figure 4.3: Water hyacinth A. canopy temperature and B. water temperature over 2 years (2004-2006) at Delta Park (blue) and Mbozambo Swamp (red). Day 1 corresponds to 01 December 2004.

Neochetina *oviposition*

Oviposition data was drawn from DeLoach and Cordo (1976; Table 3.3). *Neochetina eichhorniae* oviposition rates were much higher between 25-30°C, but decreased above 30°C (Figure 4.4). Using the oviposition rates described by DeLoach and Cordo (1976), temperature-dependent oviposition was described as in (Equation 27).

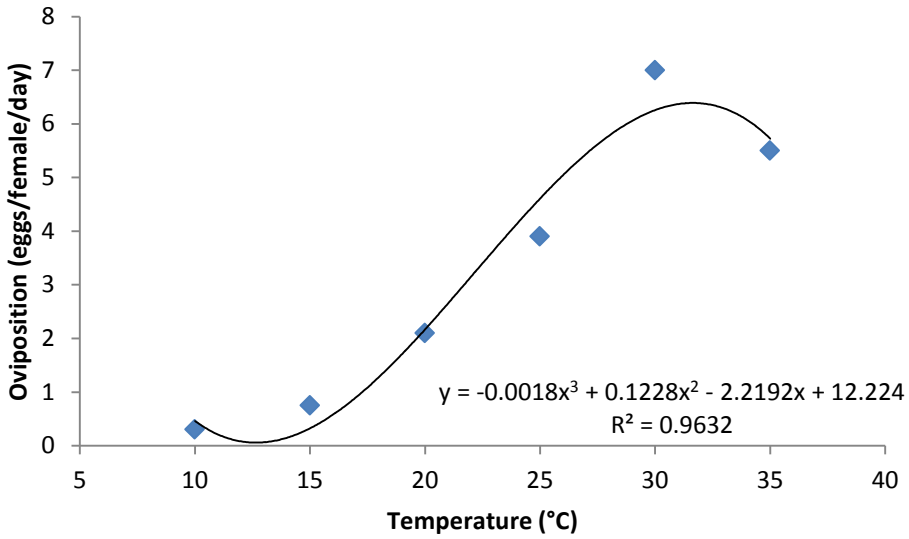


Figure 4.4: The effect of temperature on the oviposition of *Neochetina eichhorniae* at five constant temperatures (data from DeLoach and Cordo, 1976). The solid line represents the third order polynomial regression.

Introduced temperature-dependence resulted in variable oviposition rates between sites (Figure 4.5). On average, modelled oviposition rates for Mbozambo Swamp were 2.45 eggs/female/day higher than at Delta Park, which would lead to larger egg populations.

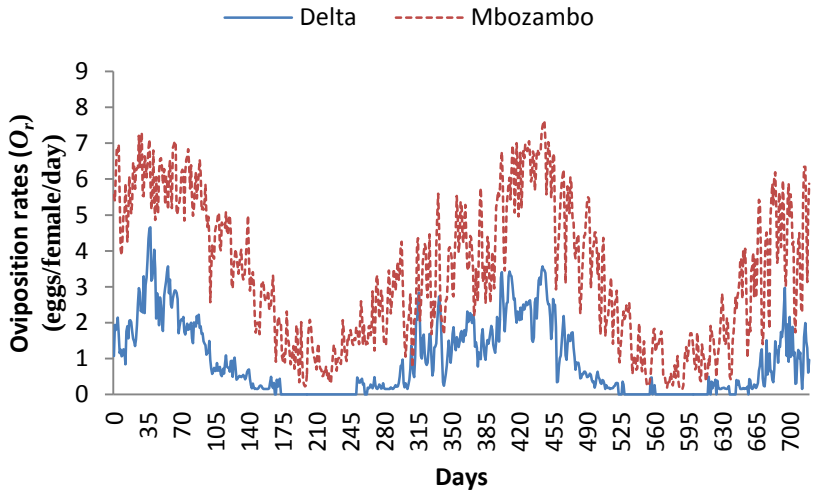


Figure 4.5: Modelled temperature-dependent oviposition (O_r) at Delta Park (blue) and Mbozambo Swamp (red) for Stage 2 (temperature-dependent) models. Day 0 corresponds to 01 December 2004.

Insect survival

Egg survival proportions (S_E) were, on average, 0.38 times higher at Mbozambo Swamp (mean S_E =0.66 ± 0.21 SE) than at Delta Park (mean S_E =0.28 ± 0.29 SE; Figure 4.6). At Delta Park, egg survival was zero for 317 of 730 days while at Mbozambo only 12 out of 730 days had zero egg survival. This would result in denser egg and subsequently larval populations (Figure 4.6).

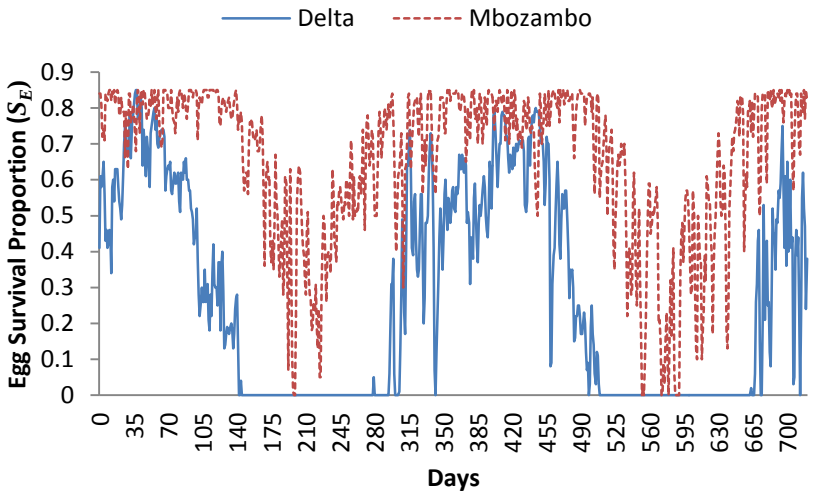


Figure 4.6: Modelled temperature-dependent egg survival proportion (S_E) at Delta Park (blue) and Mbozambo Swamp (red) for Stage 2 (temperature-dependent) models. Day 0 corresponds to 01 December 2004.

Weevil populations

Populations for all immature weevil life stages occurred at higher densities at Mbozambo Swamp than at Delta Park (Figure 4.7). Larval densities increase to over 16 000 larvae/m^2 compared to less than 8 000 larvae/m^2 at Delta Park. Both sites maintained adult weevil populations at the carrying capacity of 150 weevils/m^2 for the duration of the modelling cycle. Higher temperatures at Mbozambo Swamp allowed faster development, with the first (F_1) generation of adults occurring on day 70 after the introduction of weevils. F_1 generations at Delta Park emerged over a month later on day 118 (Figure 4.7).

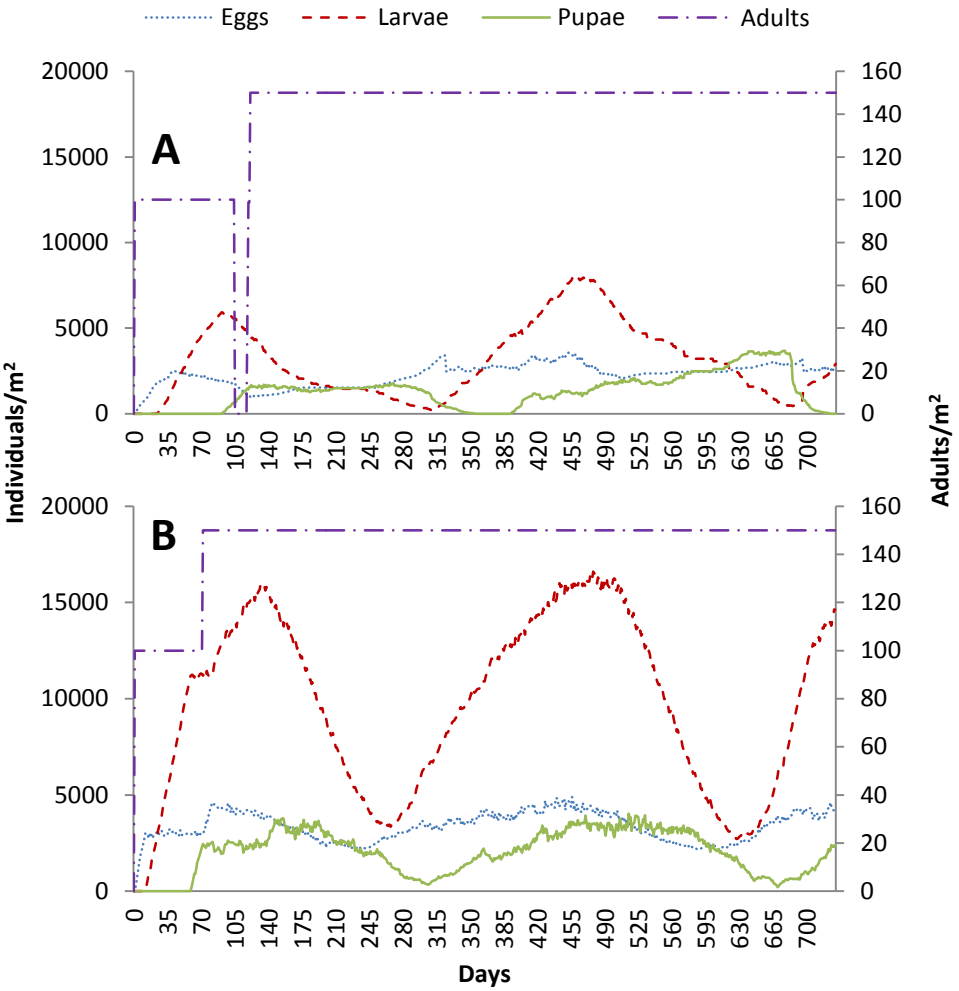


Figure 4.7: Stage 2 (temperature-dependent) *Neochetina eichhorniae* model population densities (individuals/m^2) of egg (blue), larval (red), pupal (green) and weevil adult (purple) life stages for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site). Stage 2 models include temperature-dependent oviposition rates and egg survival proportions. The scale differs on the y-axes.

Stage 3 (winter mortality) insect models

Introducing age-dependent oviposition distinctly changed the population profiles at both sites (Figure 4.8). Oviposition only occurs for the first 21 days of a female weevil's adult life, resulting in distinct generations within the weevil populations. At Delta Park, only three subsequent generations occurred during the two-year modelling cycle. These adult populations only increase to 300 weevils/m^2 for short periods when young and old weevil populations overlap (days 131-160; 679-711). Egg and larval populations are smaller than in Stage 2 (temperature-dependent) models, reaching maximums of 2615 eggs/m^2 and 2295 larvae/m^2

respectively (Figure 4.8 A). Winter mortality reduces the egg population around day 222 when minimum temperatures drop below 0°C (Figure 4.9).

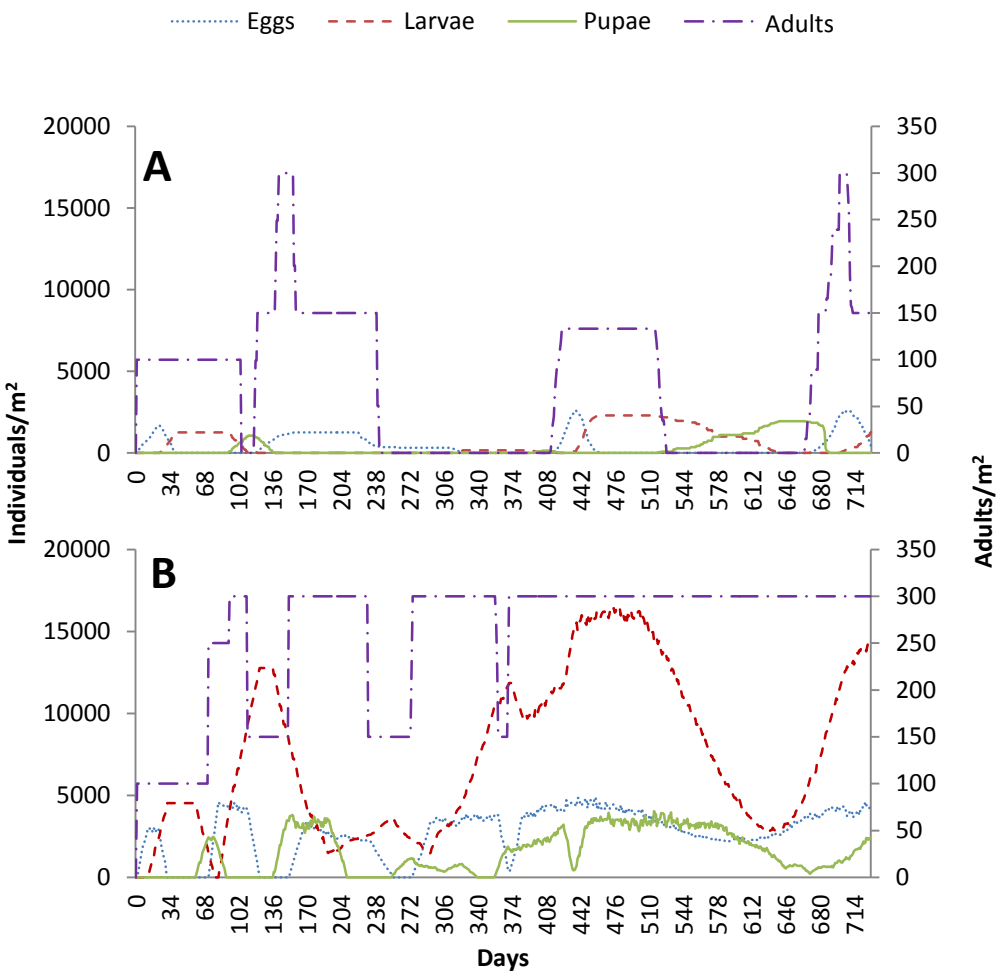


Figure 4.8: Stage 3 (winter mortality) *Neochetina eichhorniae* model population densities (individuals/m^2) of egg (blue), larval (red), pupal (green) and weevil adult (purple) life stages for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site). Stage 3 models include temperature-dependent oviposition rates and egg survival proportions, egg and larval winter mortality, and age-dependent oviposition. The scale differs on the y-axes.

At Mbozambo Swamp, however, three subsequent generations occurred within the first year, leading to a continuous overlap of generations, young and old adult weevils by early in the second year (day 371). Egg and larvae populations remained high, with larval populations remaining above 1000 larvae/m^2 after day 84 (Figure 4.8 B). No winter mortality occurs at Mbozambo Swamp, as minimum canopy temperatures never dropped below 7°C (Figure 4.9).

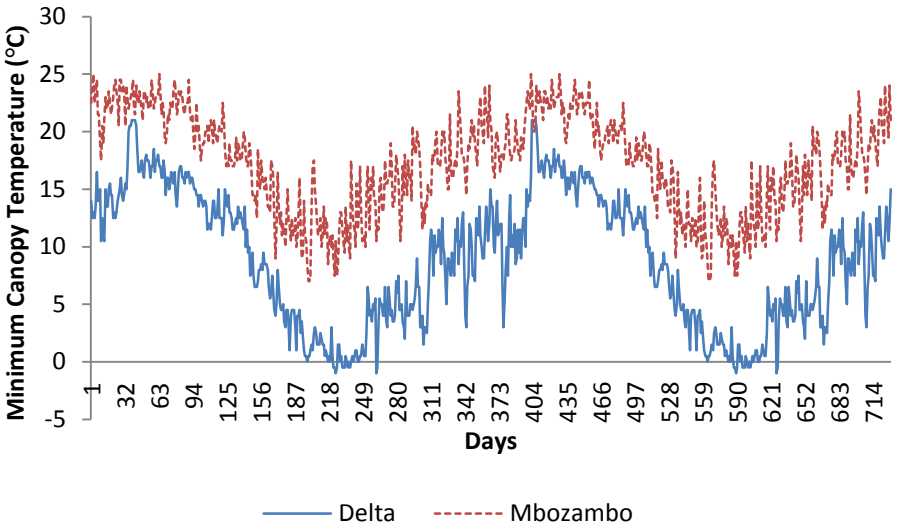


Figure 4.9: Minimum water hyacinth canopy temperatures over 2 years (2004-2006) at Delta Park (blue) and Mbozambo Swamp (red) (Byrne *et al*., 2010*)*.

Combined models of water hyacinth biocontrol

Neochetina eichhorniae *feeding*

Adult feeding

The effect of temperature on feeding varied depending on which source of data was used (Figure 4.10) but generally, feeding rates increased up to a maximum (30°C) before declining. The relationship used to approximate feeding in Stage 4 models showed a good fit to the data (R^2=0.8084) and was described by the curve,

$$y = -0.0114x^3 + 0.6394x^2 - 5.4516x$$

(Equation 41)

where *y* is the adult feeding rate (mm^2/weevil/day) and *x* is the temperature. The adult feeding rate at 25°C was approximately 85.21 mm^2/weevil/day using the above equation.

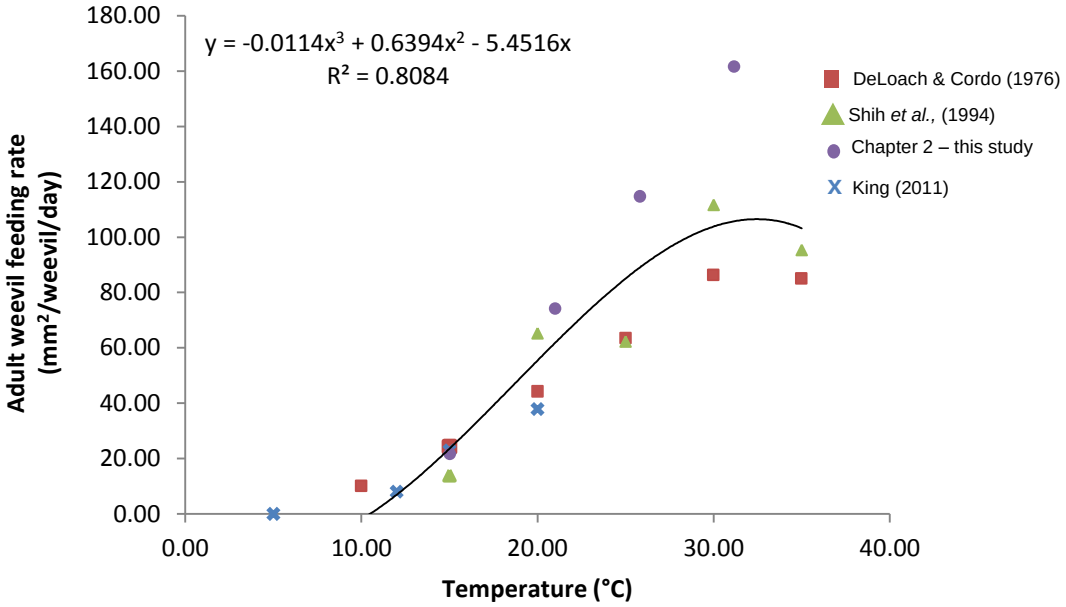


Figure 4.10: The effect of temperature on *Neochetina* adult weevil feeding. Data that was expressed in scars was converted to mm^2 using a conversion of $4.5mm^2$/scar. The solid line represents a third order polynomial regression of adult weevil feeding rate against temperature.

Larval feeding

Larval feeding at 25°C was 0.9 g/larvae/day, using the adult temperature-dependent feeding equation combined with a conversion factor (L_f) produced the larval temperature-feeding relationship

$$y = -0.0001x^3 + 0.0068x^2 - 0.0576x - 2 * 10^{-14}$$

(Equation 42)

where y is the larval feeding rate (g/larval/day) and x is the temperature. Maximum larval feeding is expected to occur at 30°C at a rate of 1.10 g/larva/day while no feeding is expected below approximately 10.5°C (Figure 4.11).

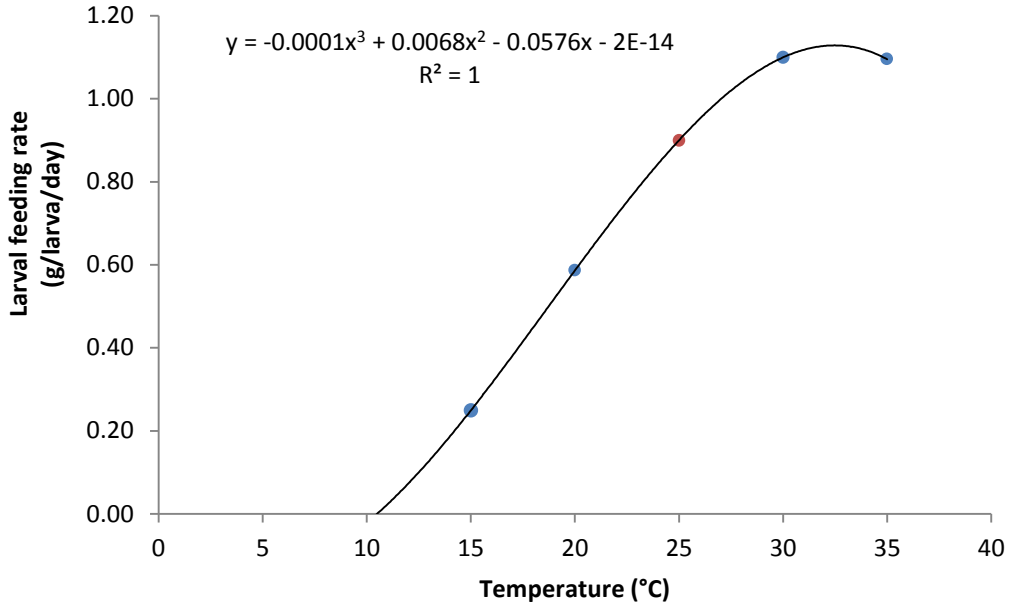


Figure 4.11: The effect of temperature on *Neochetina* larval weevil feeding. Larval feeding was measured at 25°C (red). All other temperatures were estimated using the adult temperature-dependent feeding relationship and a larval conversion factor (L_f)

Plant and insect model integration

Stage 4 (integrated) model

When temperature-dependent feeding was incorporated into the water hyacinth growth models (Stage 4 (integrated)), simulated water hyacinth densities were much more variable than when no biocontrol was included (Figure 4.12). Simulated water hyacinth populations at Delta Park varied over the two-year simulation, reaching a maximum of ca. 67 kg/m^2 during the second year (Figure 4.12 A). Declines in the water hyacinth population occurred when larval feeding increased around day 34 and day 442, corresponding to larval population maxima (Figure 4.8 A).

At Mbozambo Swamp, however, water hyacinth populations declined to zero in the first 33 days. Extremely high larval biomass removal occurred (Figure 4.12 B) as a result of very high larval population numbers (Figure 4.8 B).

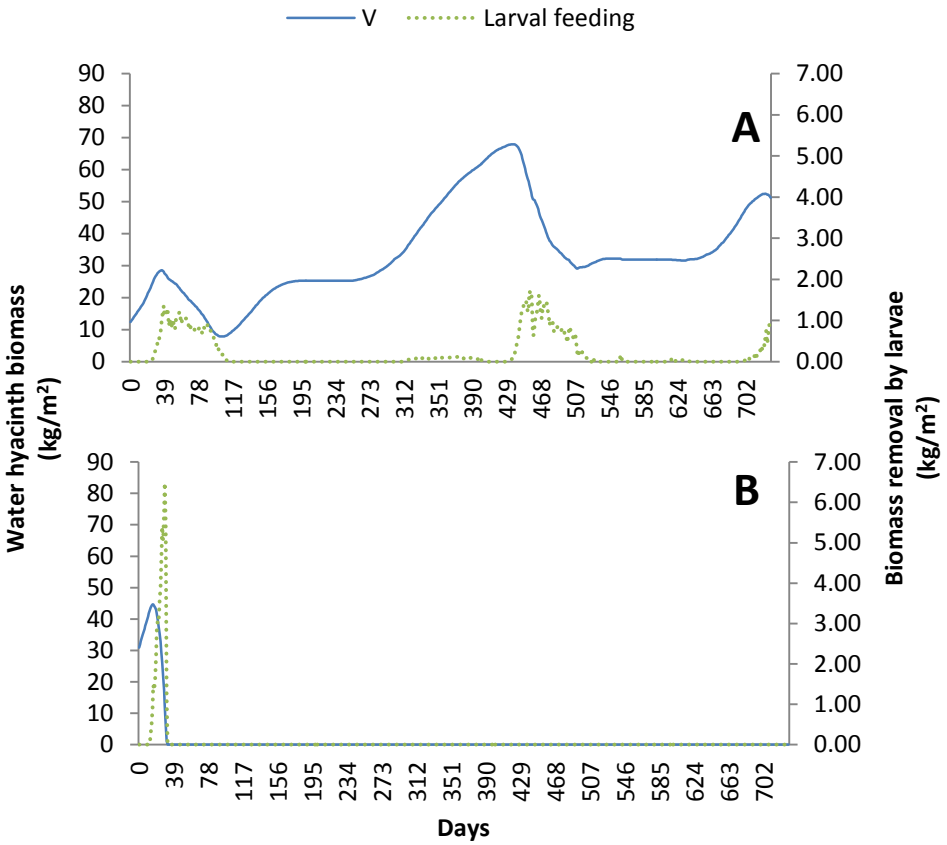


Figure 4.12: Stage 4 (integrated) model population densities of water hyacinth (*V*) and *Neochetina* larval plant biomass removal (*Larval feeding*). Water hyacinth populations were modelled under temperature-driven feeding by *Neochetina eichhorniae* adults and larvae for A. Delta Park (cold site) and B. Mbozambo Swamp (warm site).

Adult *Neochetina* feeding was also simulated at both Delta Park and Mbozambo Swamp sites (Figure 4.13). Adult biomass removal remained low (maximum of ca. 0.005 kg/m^2) throughout the simulation period for Delta Park, particularly in winter months when no feeding occurred at all. Adult biomass removal at Mbozambo Swamp was equally low, and did not extend past day 33 (Figure 4.13), once water hyacinth populations were extinct (Figure 4.12 B).

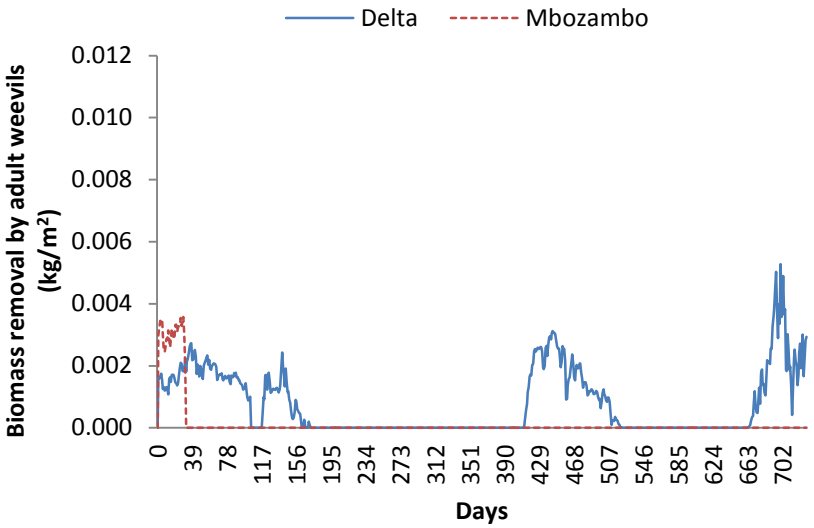


Figure 4.13: Stage 4 (integrated) model – simulated *Neochetina* adult weevil plant biomass removal for Delta Park (blue) and Mbozambo Swamp (red). Compare with Figure 4.12 to see the difference in the scale of larval to adult plant biomass removal.

Stage 4b (larval carrying capacity) model

Because of the extremely high larval population densities in Stage 4 (integrated) models, a larval carrying capacity (carrying capacity) was instituted in Stage 4*b* (larval carrying capacity) models. Larval populations reached the carrying capacity at Delta Park, but they declined during winter months and were not sustained at carrying capacity for the entire modelling period as they were at Mbozambo Swamp (Figure 4.14 A, B). Limiting the larval populations resulted in a decrease in larval biomass removals for both sites (Figure 4.14C, D), with maximum plant biomass removal of 0.59 and 1.0 kg/m^2 at Delta Park and Mbozambo Swamp respectively. Water hyacinth populations, particularly at Mbozambo Swamp, did not decline as rapidly as in Stage 4 (integrated) models, instead the weed's populations were sustained at high densities (53-69 kg/m^2 at Delta Park; 48-62 kg/m^2 at Mbozambo Swamp).

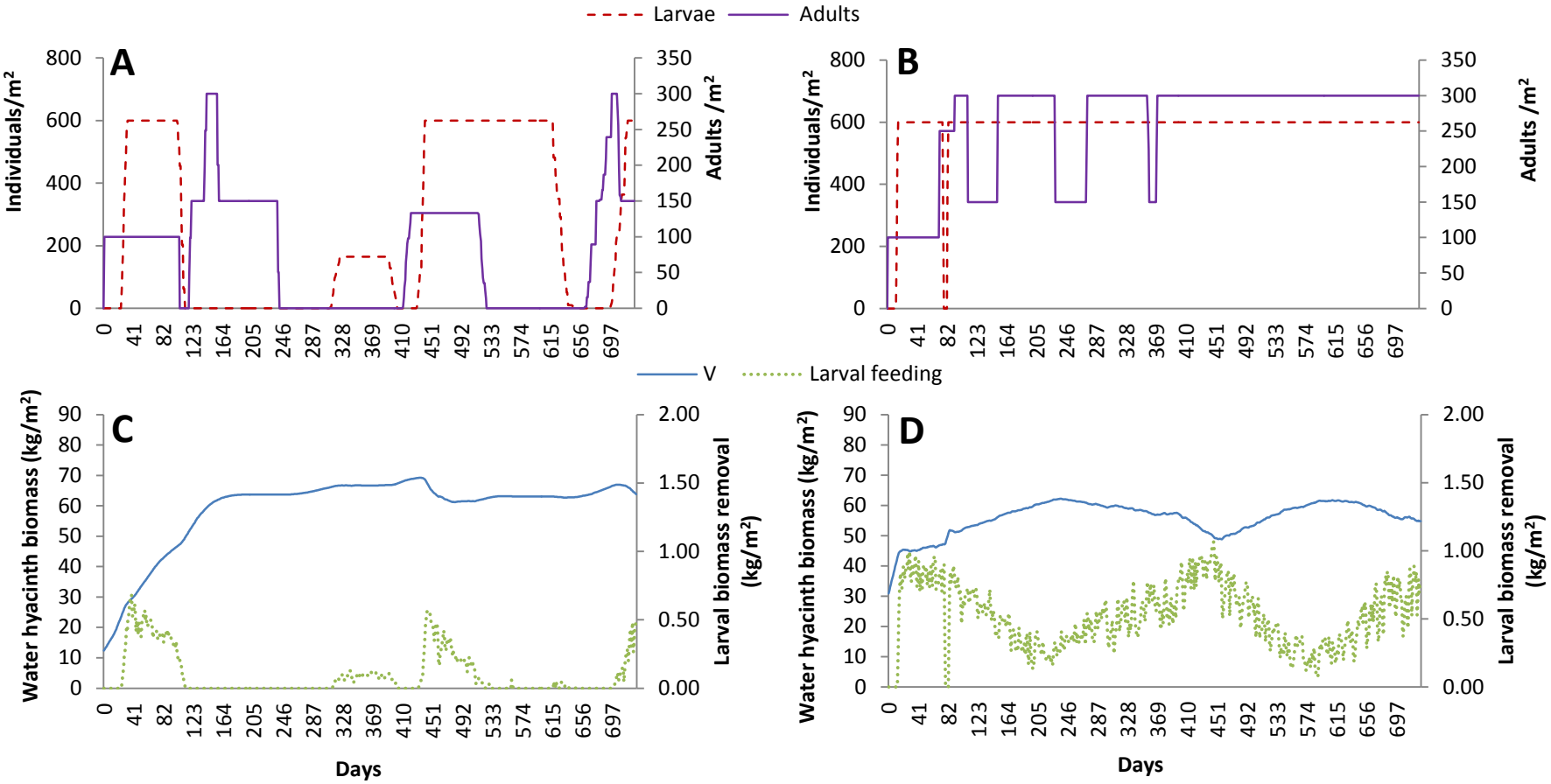


Figure 4.14: Stage 4*b* (larval carrying capacity) model population densities of *Neochetina* adults (purple) and larvae (red), water hyacinth (*V*, blue) and larval plant biomass removal (green). Weevil and water hyacinth populations were modelled for A & C. Delta Park (cold site) and B & D. Mbozambo Swamp (warm site).

Simulated adult weevil plant biomass removal between Stage 4 (integrated) and Stage *4b* (larval carrying capacity) models did not change for Delta Park. Without the extinction of water hyacinth populations caused by larval feeding in the Stage 4 (integrated) model, biomass removal at Mbozambo Swamp for the Stage *4b* (larval carrying capacity) model increased, reaching a maximum of ca. 0.011 kg/m^2 (Figure 4.15). Adult plant biomass removal at Mbozambo Swamp was sustained over the two-year modelling period, fluctuating with seasonal temperatures, but did not stop, even during the winter months.

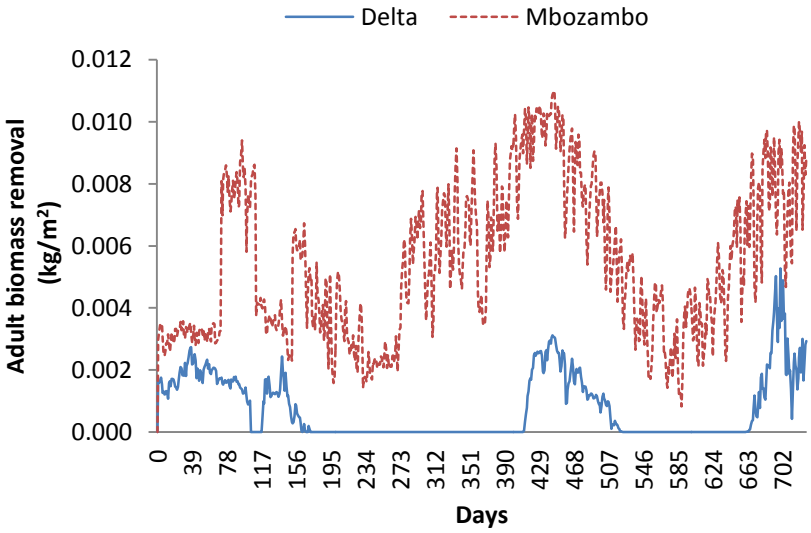


Figure 4.15: Stage 4*b* (larval carrying capacity) model – simulated *Neochetina* adult weevil plant biomass removal at Delta Park (blue) and Mbozambo Swamp (red).

4.3.2 Model validation

Comparison to field sites

Generally, Stage 4*b* (larval carrying capacity) models simulated all populations to be larger than those populations observed in the field, at both Delta Park and Mbozambo Swamp (Figure 4.16).

Modelled water hyacinth biomass was significantly larger than observed biomass in the field for both sites (Delta Park: $F_{7, 32} = 10.543$; $p<0.01$; Mbozambo Swamp: $F_{7, 32} = 11.131$; $p<0.01$; Figure 4.16 A, B). Similarly, modelled larval populations were significantly different to observed larval population for Delta Park ($F_{7, 32} =$

4.2096; $p<0.003$), being both larger and smaller than observed populations, depending on the season. Although the data source*season interaction was not significant for larval populations at Mbozambo Swamp ($F_{7, 32} = 2.3$; $p>0.05$), the mean modelled larval population (mean 240 larvae/m^2) was significantly higher than observed larval populations in the field (mean 86 larvae/m^2; $F_{1, 32} = 343.01$; $p<0.01$; Figure 4.16 D).

Tukey HSD post-hoc test, however, revealed that the modelled larval populations for Mbozambo Swamp were significantly greater than field estimates each season.

Means of modelled and observed adult population were significantly different at Delta Park ($F_{1, 32} = 9.2983$; $p<0.005$), generally overestimating population sizes, but the interaction between source and season was not significant ($F_{7, 32} = 1.9084$; $p>0.1$). For Mbozambo Swamp, however, the source*season interaction ($F_{7, 32} = 8.7534$; $p<0.001$) was significant and the modelled population means were significantly greater than observed population means ($F_{1, 32} = 123.6$; $p<0.01$). Observed adult populations at Mbozambo Swamp and Delta Park peaked around day 60 and day 150 respectively, where models predicted peaks around day 90 and day 150 respectively (Figure 4.16 E, F). Similar to larval populations at Delta Park, adult numbers were underestimated during the winter months of the second year (Figure 4.16 C, E).

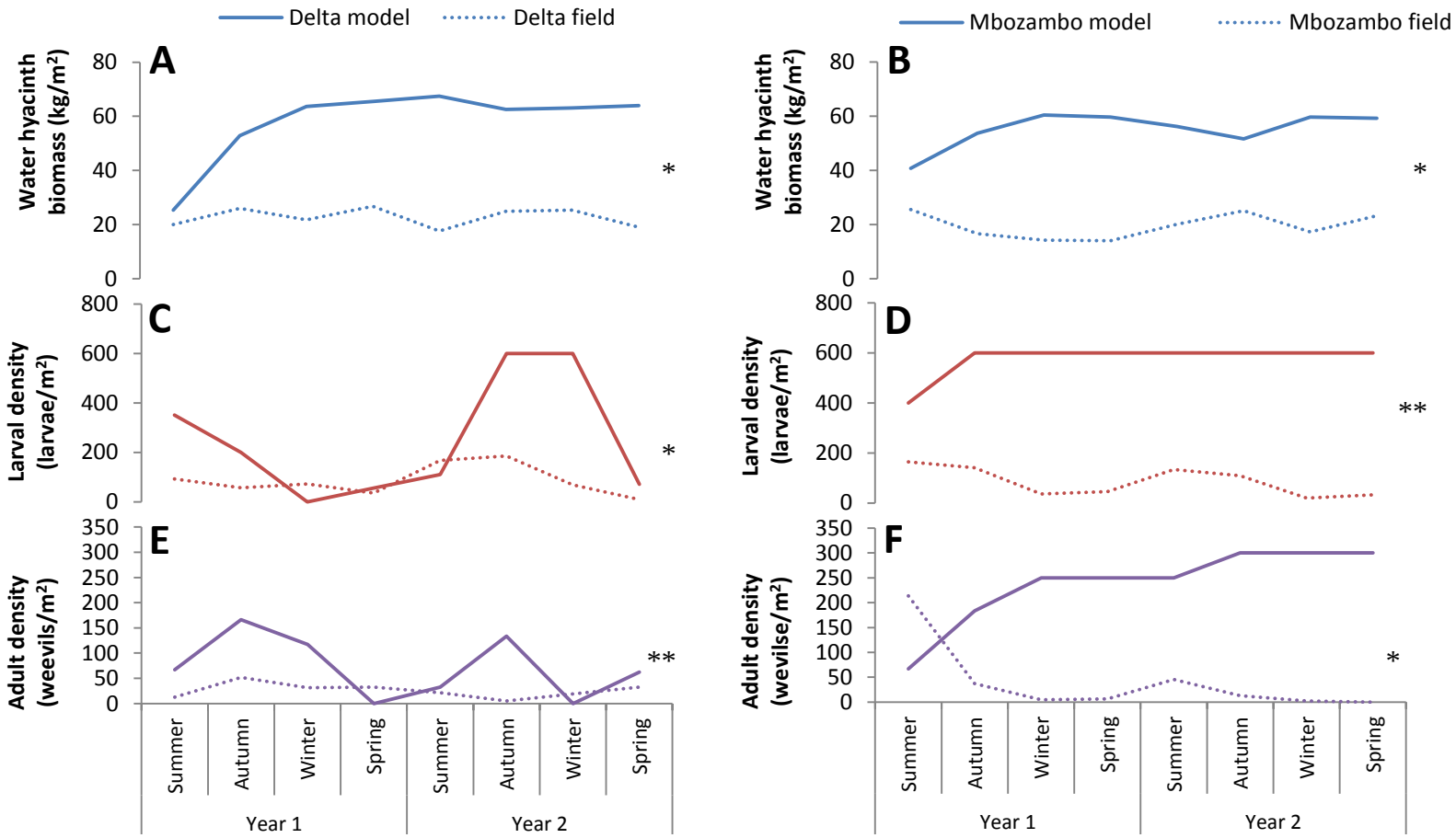


Figure 4.16: Comparison between Stage 4*b* (larval carrying capacity) modelled populations and field populations for Delta Park (A, C, E) and Mbozambo Swamp (B, D, F). Water hyacinth (blue), larval (red), and adult weevil simulated populations (purple) are shown by solid lines; all field populations are shown by dotted lines. * Significant interactions (source*season) ** Significant difference in means

4.4 Discussion

4.4.1 Water hyacinth models

Water hyacinth population growth has often been described by the logistic growth equation, and is therefore used extensively in modelling water hyacinth populations (Mitsch, 1976; Aoyama and Nishizaki, 1993; Gutierrez *et al.,* 2001; Mahujchariyawong and Ikeda, 2001; Wilson *et al.,* 2001; Wilson *et al.,* 2005). The Stage 1 (excluding temperature) model (Figure 3.2) built in STELLA displayed a typical response of the logistic growth curve (Figure 4.1) (Silvertown, 1987; Tsoularis and Wallace, 2002), where the population increased almost linearly, until it approached the system carrying capacity (*K*).

Based only on the logistic equation (Equation 4), the response of the water hyacinth model would be directly affected by the growth rate of the population. In reality, however, the population growth rate is, amongst other factors, influenced by changes in temperature. Fluctuations in temperature alter the rates of chemical reactions such as photosynthesis and respiration (Carr *et al.,* 1997), which in turn influence the rate of water hyacinth growth. The Stage 2 (temperature) models thus incorporated variable water temperature taken from the field (Figure 3.3; Byrne *et al.,* 2010), but the model response did not change drastically under either of the two different temperature regimes and both models continued to exhibit normal logistic growth behaviour (Figure 4.2; Silvertown, 1987; Tsoularis and Wallace, 2002). Despite growth rates at Delta Park being generally lower than those at Mbozambo Swamp and declining just below zero during winter months, the water hyacinth population still reached the carrying capacity. Prevailing temperatures, even in winter months, do not result in negative growth rates, which would lead to water hyacinth population declines. If the modelled relationships suggested in two freshwater systems representing the extremes of temperature in which water hyacinth occurs hold, temperature is unlikely to limit water hyacinth population in in South Africa.

4.4.2 *Neochetina eichhorniae* models

All models were run for two sites in South Africa which were representative of warm (Mbozambo Swamp) and cold sites (Delta Park) across the country (Byrne *et al.*, 2010). The canopy and water temperatures were significantly different for each site with mean daily canopy and water temperatures of only ca. 14°C at Delta Park and ca. 21°C and 25°C respectively at Mbozambo Swamp (Figure 4.3). Additionally, mean daily canopy and water temperatures minimums were between 3.9-5.4°C at Delta Park and 11.9-15.4°C at Mbozambo Swamp (Figure 4.3). Differences in temperature regimes have important implications for growth of both *Neochetina* and water hyacinth populations, as both plant and insect populations are dependent on temperature to some extent (DeLoach and Cordo, 1976; Del Fosse, 1977; Cherrill and Begon, 1989; Carr *et al.*, 1997; Chikwenhere, 2000; van der Heide *et al.*, 2006). *Neochetina* development is determined by the available degree-days above the species' developmental threshold (Herms, 2004; King, 2011). Cooler temperatures at Delta Park immediately put these insect populations at a disadvantage, as development cannot be completed as quickly as at warmer sites. Reductions in the rate of insect development and subsequent decrease in number of generations per year influence how effective biological control can be by slowing population growth (Cole, 1954; Coetzee *et al.*, 2007*b*), suggesting that biocontrol of water hyacinth at Delta Park will not be as successful as at Mbozambo Swamp.

Neochetina *oviposition and survival*

Insect oviposition and survival is directly influenced by the temperatures to which they are exposed (Higley and Haskell, 2002). DeLoach and Cordo (1976) showed that *Neochetina* oviposition varies with temperature, increasing with increasing temperatures, within an optimum temperature range (Figure 4.4). Weevils exposed to higher temperatures, within this optimal range, will oviposit more eggs in a given space of time. This was clearly demonstrated in Stage 2 (temperature-dependent) models of Mbozambo Swamp, which showed oviposition rates that were 2.45 eggs/female/day higher than rates on corresponding days at Delta Park (Figure 4.5). Models also show that at Delta Park oviposition stops during winter

months when temperatures drop below the oviposition threshold (10°C), but does not stop at Mbozambo Swamp (Figure 4.5). Consequently, consistently higher and continuous oviposition rates will lead to greater egg populations within water hyacinth/*Neochetina* systems at Mbozambo Swamp. Increased egg populations lead to larger larval and adult populations, increasing the damage potential of weevil populations at warmer sites, and potentially resulting in greater control of water hyacinth populations. Very little is known about egg populations in the field as eggs are laid between layers of water hyacinth tissue in the leaves and petioles (DeLoach and Cordo, 1976; Shih *et al.,* 1994) and are thus difficult to find and measure in the field. Verifying the modelled egg population densities is therefore very difficult.

The size of egg populations does not solely determine how effective weevil populations will be. The hatching and survival of those eggs is equally important, particularly because larvae are the most damaging life stage of *Neochetina* weevils (Chapter 2). Percent survival for almost half of the simulation period was zero at Delta Park because of the low canopy temperatures during winter, and averaged a mere 28 ± 29% (mean ± SE), while at Mbozambo Swamp only 12 zero days occurred and percent survival averaged 66 ± 21% (mean ± SE). At Mbozambo Swamp, more eggs can survive more of the time, leading to increased larval populations when compared to Delta Park. However, in reality, larval populations at Mbozambo Swamp are not always larger than populations at Delta Park, particularly in the second year (Figure 4.16).

Neochetina *populations*

Simulations of Neochetina populations at Delta Park and Mbozambo Swamp using Stage 2 (temperature-dependent) models illustrate how temperature effects on survival, oviposition and development are compounded when determining weevil populations. Although adult populations at Mbozambo Swamp and Delta Park both reached the initial carrying capacity of 150 weevils/m^2 in the F_1 generation, adult populations at Mbozambo Swamp emerge over a month earlier than at Delta Park, allowing new adults to oviposit earlier in the year

(approximately February). The delay in adult emergence at Delta Park results in new weevils ovipositing later in the year (approximately March/April), when temperatures start to decline. This means that over and above the general temperature difference between the two sites, there is also a temporal difference, which further reduces the oviposition, survival and development potential of weevil populations at a cold site (Figure 4.7). Corresponding reductions in population growth further reduces the potential for control at cold sites, as delays in the emergence of new generations of weevils allow more time for plants to grow (Byrne *et al.,* 2010). Although higher temperatures at Mbozambo Swamp allow for much larger insect population densities, particularly of larvae (Figure 4.7), they also allow for increased plant growth (Sato, 1988). If weevil populations are sufficiently large, the effects of herbivory are likely to contribute to control of the weed. However, if temperatures and other contributing factors are favourable, water hyacinth could compensate for herbivory effects (Soti and Volin, 2010).

In Stage 3 (winter mortality) models, temperature-dependent oviposition only occurred in the first 21 days of the adult life span, reducing the simulated egg populations for both sites (Figure 4.8). For Delta Park, population profiles are distinctly different to those in Stage 2 (temperature-dependent) models (Figure 4.7). Distinct adult generations emerged, particularly in the warmer months (days 0-140; 315-525; 665-730; Figure 4.8 A). In the first year, only one (F_1) generation occurred, but in the second year, two generations of new adults were simulated. Similarly, Byrne *et al.*, (2010) estimated that approximately two generations of weevils occur at Delta Park each year (estimated from larval mines and degree-day calculations). In Mbozambo Swamp simulations, three generations occurred in the first year, and by the second year, generations were overlapping completely (Figure 4.8 B). If the initial population is included, generation numbers are again similar to approximations by Byrne *et al.,* (2010), who estimated 4.39 generations per year (using degree-day calculations).

Together with age-dependent oviposition, Stage 3 (winter mortality) models included additional winter mortality estimated in relation to minimum canopy temperatures (Figure 4.9). Temperatures at 0°C and below were taken to represent leaf-frosting events that could potentially kill off egg and larval populations that are dependent on leaves and petioles for their survival (Owens and Madsen, 1995; Grodowitz *et al.,* 1991). Minimum temperatures never drop below 7°C at Mbozambo Swamp, but repeatedly drop to zero or below at Delta Park, particularly between days 187-280 (05 June – 06 September) and 559-621 (12 June – 13 August) (Figure 4.9). During these periods for Delta Park, decreases in the modelled egg and larval populations occurred (Figure 4.8 A). Only low numbers of third instar larvae survived the winter, contributing to the new generation of adults in later months. The delayed development and emergence of adult weevil populations in the second year of the model may provide sufficient time for water hyacinth populations to take advantage of the warmer temperatures in an enemy free space. Because Mbozambo Swamp populations in the model have no winter mortalities (Figure 4.8 B), weevil populations are able to grow rapidly, potentially contributing to greater control of the weed at this site. However, significant control of actual water hyacinth populations at Mbozambo Swamp has not yet been achieved (Byrne *et al.,* 2010). At another site, Wewe Siphon Dam (29°32’29.41”S 31°08’07.41”E), which is close to Mbozambo Swamp control of water hyacinth populations was achieved (through augmentative release) (Conlong *et al.,* 2009). Weevil populations at Mbozambo Swamp may therefore be under the influence of other factors, such as the effects of nutrients.

4.4.3 Combined models of water hyacinth biocontrol

Neochetina eichhorniae *feeding*

Although insect models can simulate or predict populations and generations per year, to understand the control potential of an agent, its capacity to damage the host plant needs to be quantified. *Neochetina* weevils are herbivores that feed only on water hyacinth plants. Quantifying the adult and larval damage will provide insight into understanding the weevil’s potential for controlling water hyacinth.

Although a general relationship has been formulated and used in combined water hyacinth biological control models, feeding rates presented by each author vary for given temperatures (Figure 4.10). These differences may have resulted from differences in nutrient conditions, which were not explicit in many of the studies. However, Heard and Winterton (2000) and Coetzee *et al.,* (2007*a*) found that feeding rates were not significantly affected by different nutrient regimes. Another potential source of error is the scar to leaf-area conversion factor. Many researchers measure adult weevil feeding rates by the scars/weevil/day. Although scars are regularly shaped and easy to count, scar size is extremely variable, ranging from 0.5-25mm^2 (DeLoach and Cordo, 1976; Franceschini *et al.*, 2010). Using an average scar to leaf-area conversion (4.5mm^2; DeLoach and Cordo, 1976) may then result in overestimations of areas removed through herbivory. However, overestimations are not of particular concerns in weevil/water hyacinth systems, as the biomass removed per scar, or per mm^2 is negligible relative to the plant's total biomass.

Larval feeding, however, is far more damaging than adult herbivory, in terms of biomass removal (Chapter 2). Maximum larval feeding rate estimated in this study (1.1 g/larva/day) are much higher than maximum larval feeding estimates made by Wilson (2002) (0.05-0.2 g/larva/day). Parasitic fungi and soft-rot bacteria have been associated with arthropod damage of water hyacinth plants (Charudattan *et al.*, 1978), and can cause additional plant biomass loss (Coetzee *et al.*, 2009). Here, no measures to prevent fungal infection were taken and the high larval feeding rate estimates may account for both larval biomass removal and fungal and bacterial infections.

Plant and insect model integration

Stage 4 (integrated) model

Introducing biological control into the models resulted in decreased water hyacinth biomass density in both Delta Park and Mbozambo Swamp Stage 4 (integrated) model simulations (Figure 4.12). Water hyacinth populations at Delta

Park increased for the first few weeks, before larval feeding could occur. The water hyacinth densities declined after eggs had been laid and sufficient time for development and hatching had occurred, but as soon as the larvae pupated, plant densities increased quickly (Figure 4.12 A).

Adult feeding occurs for majority of the first 170 days of the simulation period (Figure 4.13), but does little to restrain plant growth, in the absence of larval populations (Figure 4.12 A). Although larval feeding influences plant growth, it does not occur during winter months, and is often reduced to very low levels. This allows the plant population to increase, taking advantage of warmer temperatures earlier in the season, when larval populations are low and new adults have not emerged (Figure 4.8 A; Byrne *et al.,* 2010).

Larval feeding rates in the Mbozambo Swamp Stage 4 (integrated) models are higher than rates for Delta Park (7 kg/m^2 cf. 1.5 kg/m^2). Such high larval feeding rates result in the extinction of water hyacinth within 33 days in the model (Figure 4.12 B). However, local extinctions in biological control systems in the field are rare. Even in very successful biocontrol programmes, plant densities may be reduced by up to 95%, but are not eradicated (Jayanth, 1988). Such high feeding rates correspond to high larval densities, which result from large egg populations (Figure 4.8 B) and high egg survival rates (Figure 4.6). Stage 4 (integrated) models use Stage 3 (winter mortality) models to estimate larval populations, which are sustained above 1000 larvae/m^2 and up to 8000 larvae/m^2 after 84 days of simulation (Figure 4.8 B). These simulated densities are particularly high when considering larval densities in South Africa (as estimated from petiole mines) are approximately 100-200 larvae/m^2. Wilson *et al.,* (2006) found that larval survival decreased with increasing larval densities. However, density-dependent survival was not included in any of the weevil models and survival was set at a constant 85%. Additionally, egg survival remains high throughout the model simulation for Mbozambo Swamp, even in winter months. Center (1987) found that in the field in Florida, larval populations occurred at approximately half the density of egg populations and third instar larvae populations were over 15 times less numerous

than egg populations, indicating high mortality between these two stages. High egg and larval survival rates in the models resulted in overestimations of both egg and larval populations.

Modelled adult populations are unaffected by changing temperatures and do not experience seasonal mortality (in winter). Predation has also been ignored, which may have a marked effect on adult populations. Overestimated adult numbers in the models further leads to high egg and larval densities contributing to large immature individual population sizes. Additionally, larval feeding rates in the models, particularly at higher temperatures, have also been overestimated (see Section 4.4.3). A combination of large larval populations and high feeding rates is likely to overestimate the impact of larval populations, resulting in water hyacinth extinctions.

Larval damage is often cited as being more important than adult damage (Wilson *et al.*, 2001; Wilson, 2002; Wilson *et al.*, 2005; Ripley *et al.*, 2008). However, very few efforts have been made to quantify larval damage (Chikwenhere, 2000) particularly in terms of biomass loss. Although robust temperature-feeding relationships for larvae have yet to be determined, feeding measured at 25°C suggests just how much more important larval feeding is compared to adult feeding. Measured larval feeding rates are over 60 times greater than maximum adult feeding rates. Maximum larval feeding estimates for *N. eichhorniae* by Wilson (2002) are also 10-57 times greater than adult feeding rates. Understanding the relative impact of the different life stages, and parameterising larval populations in models thus becomes particularly important for accurate biocontrol simulations.

Stage 4b (larval carrying capacity) model

Because of the very high larval populations in Stage 4 (integrated) models, a larval carrying capacity of 600 larvae/m^2 was instituted, based on the average number of petioles/plant and plants/m^2 across 14 sites in South Africa (Byrne *et al.*, 2010). Reducing larval population densities subsequently reduced the level of

control exhibited in water hyacinth population simulations for both Delta Park and Mbozambo Swamp (Figure 4.14). Larval biomass removal generally remained below 1.0 kg/m^2, dropping particularly low in Delta Park simulations (Figure 4.14 C). Because local extinctions of simulated water hyacinth populations no longer occurred in Mbozambo Swamp models, both adult and larval feeding was sustained throughout the year, fluctuating with seasonal temperature changes (Figure 4.14 D; Figure 4.15).

Stage 4*b* (larval carrying capacity) models suggested that Mbozambo Swamp has better potential for control by *Neochetina* weevils, as temperatures allowed for continuous oviposition (Figure 4.5) and continuous larval and adult populations that feed throughout the year (Figure 4.14 D; Figure 4.15), ultimately preventing the plant from reaching the biomass carrying capacity (Figure 4.14 D). The models showed the importance of temperature in increasing biocontrol success. Cold winters and lower temperatures prevented rapid weevil population growth, and not only slowed development (Figure 4.14 A) but also resulted in winter egg and larval mortalities and seasonal oviposition and weevil feeding (Figure 4.5; Figure 4.14 C; Figure 4.15). Plant populations were only slightly reduced at both sites, and only during summer months when larval populations were high and larval feeding occurs (Figure 4.14 A, C).

Comparison to field sites

Models generally overestimated both water hyacinth and weevil populations when compared to field data (Figure 4.16). Water hyacinth biomass simulations were significantly greater than field estimates for both sites (Figure 4.16 A, B).

In Stage 4 models, weevil feeding is only considered as biomass removed from the system. The weevil feeding rates therefore do not affect the water hyacinth population growth rates in the models. Realistically, though, both adult and larval stages of the weevil affect water hyacinth growth rates (Bashir *et al.*, 1984; Chikwenhere, 2000). Venter *et al.*, (2013) showed that weevil-borne microbes contributed as much to water hyacinth photosynthetic rate declines as did the

removal of biomass by surface-sterilised weevils. Reductions in photosynthetic rates and productivity will likely result in reduced plant growth. Considering only biomass removal and not the additional effects of herbivory on plant growth rates therefore oversimplifies the plant/insect interaction and underestimates the overall effects of herbivory, leading to overestimations in water hyacinth populations. Further experimentation to determine the effects of weevil herbivory on plant growth (not just biomass removal) under different temperatures is needed but has not been considered, despite the weevil being used as a control agent of water hyacinth for over 40 years (Cilliers, 1991).

Models generally overestimated larval populations. As discussed earlier, model larval survival rates were constant and high. Survival of larvae is likely to follow a similar temperature-dependent relationship as in egg survival, resulting in high mortality rates in the autumn and winter months, particularly at cold sites. So far, this has not been included in models. Furthermore, field populations of larvae are half as large as egg populations in Florida (Center, 1987). Although egg survival has been made temperature-dependent in the models, it is likely still too high resulting in grossly overestimated larval populations, particularly at warm sites.

Although still inaccurate, weevil populations at the cold site (Delta Park) appear to match field populations better than at the warm site (Mbozambo Swamp). The improved performance of models at cold sites may result from increased mortality rates, leading to better estimations of weevil populations. The differential performance of the model between sites of different temperature regimes also draws attention to additional factors that may be limiting weevil populations at warm sites where agents have the potential to reach very high numbers. Perhaps predation of the adult population is prevalent at warmer sites. Water hyacinth mats are filled with potentially predatory insects, specifically very large spiders (personal observation). These spiders may predate on adult weevils, resulting in decreased adult populations and subsequently decreased control of the weed. Additionally, birds may feed on the adult weevils. Hadeda ibis have been observed feeding on weevils in pools of water hyacinth at the University of the

Witwatersrand (personal observation). Other bird species may also take advantage of these weevil populations, particularly in dense water hyacinth infestations where birds are able to sit on the floating plants.

These models do not include the effects of nutrients on either plant or weevil populations. Although most sites in South Africa are eutrophic (Byrne *et al.*, 2010; Coetzee and Hill, 2012) and should stimulate plant and weevil population growth (Reddy *et al.*, 1990; Heard and Winterton, 2000), the interaction effects of temperature and nutrients are not known and may influence these populations in unexpected ways.

4.5 Conclusion

Accurate models of water hyacinth/weevil populations remain elusive, with consistent overestimations of both populations. Although overestimations in weevil populations should result in underestimations of water hyacinth populations, water hyacinth biomass still reached carrying capacity suggesting that the effects of weevil herbivory have been underestimated by considering only plant biomass removal. Incorporating the additional effects of weevil herbivory on plant growth rates is likely to lead to better water hyacinth population estimations.

Model building helps formalise the existing knowledge about a system and helps develop hypotheses for follow up work. Modelling this system of water hyacinth and *Neochetina eichhorniae* weevils has drawn attention to the lack of specific knowledge about *Neochetina* larvae, particularly feeding rates and quantifiable damage. Further study into how feeding damage influences both growth rate and biomass removal of the plant as well as the incorporation of more realistic survival rates and additional environmental factors, such as nutrients, in both water hyacinth and weevil populations will aid the development of effect biological control models. Issues raised here will be incorporated into future modelling attempts.

5 CHAPTER FIVE – GENERAL DISCUSSION

5.1 Modelling systems

Models are simplifications of complex systems. They are developed to simulate systems in order to answer questions and increase current understanding, in a more cost effective and time efficient manner. Models also serve to formalise current knowledge and raise key issues about the systems. While no model is perfect, most models can enlighten us on the deeper workings of the modelled system.

Here, a combined water hyacinth biological control model has elucidated the importance of temperature in biological control systems. Low canopy or air temperatures affect plant populations through frost events resulting in leaf browning and leaf death (Owens and Madsen, 1995). These events, however, do not necessarily result in water hyacinth population declines. Growth of these populations is temperature-dependent (Sato, 1988), but water temperatures need to drop below 8-10°C to stop water hyacinth growth (Gopal, 1987) and only acute exposure to temperatures below -16°C or chronic exposure (two to three weeks) to temperatures below 5°C results in water hyacinth stem-base mortality (Owens and Madsen, 1995), causing population declines. Temperatures at warm sites in South Africa often remain well above thermal minima for water hyacinth growth and at cold sites rarely drop so low, preventing significant water hyacinth population declines**.** Minimal stem-base mortality during winter months allows water hyacinth to take advantage of increasing temperatures very early in the growing season resulting in rapid increases in water hyacinth biomass. Furthermore, any leaf mortality that does occur within the water hyacinth population contributes to the decomposing matter in the upper water surfaces that releases nutrients (Gupta *et al.,* 1996). Increased water nutrient concentrations accessible to the plant populations are likely to further increase plant growth (Reddy *et al.,* 1989; Reddy *et al.,* 1990) contributing to plant resurgence in spring and summer.

Insect populations, however, are much more sensitive to temperature. Cold winters at some South African sites severely limit weevil population growth. Developmental rates are slower and low minimum temperatures and frosting causes leaf senescence (Owens and Madsen, 1995), which decreases the survival of weevil eggs and early instar larvae located in the leaves and upper petioles of water hyacinth plants (DeLoach and Cordo, 1976; Grodowitz *et al.*, 1991). Winter populations are reduced to late larval instars and pupae, which often have to overwinter in the plant stolon and roots. At the onset of spring, new adults take longer to emerge at cold sites because of reduced amount of developmental heat available in winter and early spring months. Furthermore, oviposition rates are lower at lower temperatures (DeLoach and Cordo, 1976), meaning that contributions by new adults to the next generation are limited, further decreasing the potential population density. Low oviposition rates and survival and slow developmental times result in fewer generations per year and low population densities (Heard and Winterton, 2000). Larger agent populations have been shown to exert greater control over water hyacinth populations (Center *et al.*, 1999*b*; Bownes *et al.*, 2010*b*). Consequently, low temperatures disadvantages weevil agents, reducing the potential control weevils may have at colder sites.

Models also raise key issues around larval population densities. Field larval populations in South Africa are approximately 100-200 larvae/m^2, yet in models larval populations increase to 600 larvae/m^2 or up to 16 000 larvae/m^2 (when no carrying capacity is included). This begs the question, why are field larval populations not so high? Mortality is the most probable answer. Modelled larval populations experience constant mortality of 85% when moving into the next life stage. In reality, however, mortality will occur as individuals move between the different instars (Center, 1987) and will be influenced to some extent by the prevailing temperature conditions. Larval survival is likely to follow a similar temperature-dependent relationship as egg temperature-dependent survival and both will be influenced by leaf senescence and turnover.

Weevil eggs are oviposited in older leaves (Center, 1987), but at high temperatures water hyacinth plants can produce new leaves every 5-14 days (Center and Spencer, 1981; Byrne *et al.*, 2010; Hill, 2014). Each week, older leaves are displaced and leaf deterioration occurs (Center, 1987) which may cause increased mortality of eggs and early instar weevil larvae. Furthermore, in the winter, frost events cause leaf browning (Owens and Madsen, 1995; King, 2011) increasing leaf mortality and subsequently egg and larval mortality. These additional sources of mortality, beyond just temperature-effects, will result in lower egg and larval population densities. Additionally, adult weevil populations will be influenced by canopy temperature. Adult longevity varies with prevailing temperatures, ranging from 10-39 days at 28°C ± 2°C (Oke, 2008) to 112 days at 21-24°C (Njoka *et al.*, 2006). Differences in adult longevity would affect adult population densities. Models assume constant adult weevil longevity of 104 days, with oviposition occurring in the first 21 days. If high temperatures actually decrease adult longevity to less than 21 days, model oviposition approximations would be overestimated, further contributing to high egg and larval population densities.

5.1.1 Food for thought

With larvae being the most damaging life stage of *Neochetina* weevils (Bashir *et al.*, 1984), such overestimated model weevil populations should result in underestimations of water hyacinth population densities. However, this was not the case, so why are the model weevils not damaging the plants?

In the model, by the time larvae had begun to hatch (14-21 days) plant populations had increased to approximately 26 and 42 kg/m^2 at Delta Park and Mbozambo Swamp, respectively. With plant population growth rates of 0.04-0.05 g/g/day, water hyacinth populations are more than able to compensate for simulated *Neochetina* feeding. Compensatory growth is common in plant/herbivore systems. Hare (1980) found that defoliation by the Colorado potato beetle during the first three weeks of the growing season had little effect on potato yields while Watt *et al.*, (2007) found that 66-100% defoliation of the weed

Buddleia davidii by the weevil *Cleopus japonicus* increased aboveground biomass. Additionally, Soti and Volin (2010) showed that 10% defoliation through simulated weevil herbivory did not result in any difference in biomass allocation and relative growth rate in water hyacinth.

However, simulated herbivory underestimates the influence of *Neochetina* weevils. Venter *et al.,* (2013) showed that approximately half of the reductions in photosynthetic rates caused by *Neochetina* weevils could be attributed to the effect of microbes, which are introduced during the feeding process. Furthermore, Bashir *et al.,* (1984) showed that *Neochetina eichhorniae* larvae and adult males could reduce the growth rate of water hyacinth by 28.6% and 4.3%, respectively. A potential 30% reduction in water hyacinth growth is likely to influence how the plant responds to herbivory as well as the level of control achieved at a particular site. Incorporating the influence of herbivory as reductions in water hyacinth growth as well as biomass removal is likely to yield more accurate estimations of water hyacinth populations.

Although overall weevil damage has been underestimated, quantified larval damage was too high (0.9 g/larva/day). *Cornops aquaticum*, a grasshopper that has been released against water hyacinth in South Africa, removes biomass at a rate of 0.029 to 0.119 g/individual/day, depending on the life stage (nymph vs. adult) (Franceschini *et al.,* 2011). This is seven to 30 times less biomass than *Neochetina* larvae estimates made here. However, arthropod damage on water hyacinth plants, particularly *Neochetina* weevils, has been associated with soft-rot bacterial and fungal infections (Charudattan *et al.,* 1978; Venter *et al.,* 2013) which contribute to biomass loss. The high weevil larvae feeding rate probably accounts for the plant biomass loss by both weevil larvae and plant infections. Temperature effects on larval damage are also poorly understood. Here a temperature-dependent relationship of larval feeding was approximated, assuming that larvae follow the same temperature-dependence pattern as adult weevils. This temperature-dependence will need to be verified in order to determine the effects of larval damage under changing environmental temperatures. Understanding and

quantifying the effects of frost on both weevil survival and feeding will also contribute to better estimations of weevil populations and hence control of water hyacinth.

Although plant quality or environmental nutrients have no effect on insect feeding rates, nutrients significantly influence the level of control achieved over water hyacinth plants (Heard and Winterton, 2000; Coetzee *et al.*, 2007*a*). Heard and Winterton (2000) showed that *Neochetina eichhorniae* weevil herbivory at medium water nutrient levels (1.4 mg/L NO_3-N and 0.025 mg/L PO_4-P), and subsequently plant nitrogen and phosphorus levels of 3% and 0.2% respectively, resulted in greater water hyacinth biomass loss and fewer ramets per plant compared to high nutrient levels. Water systems in South Africa, however, are generally eutrophic (2.5-10 mg/L N; 0.025-0.25 mg/L P) (DWAF, 1996; Byrne *et al.*, 2010), stimulating water hyacinth growth (Reddy *et al.*, 1989; Reddy *et al.*, 1990; Heard and Winterton, 2000). While high water nutrients promote water hyacinth growth, they potentially reduce control by *Neochetina eichhorniae* weevils. Furthermore, temperature and nutrients can interact to influence insect feeding and growth (Lee and Roh, 2010), but the effect of this is not known in *Neochetina* weevils. To determine the influence of *Neochetina eichhorniae* as a biological control agent of water hyacinth, such nutrient and temperature effects will have to be considered.

5.1.2 The ideal model

Building the ideal model of water hyacinth biological control would need to incorporate interactions between both water hyacinth and weevil populations. Currently, weevil models influence plant models through herbivory, but plant models have no effect on the weevils. This effect can be achieved by incorporating nutrients into the model system. Plant nutrients influence the fecundity and subsequently the population growth of *Neochetina* weevils (Center and Dray, 2010*a*). If nutrients and their effects on both plant and weevil population growth can be quantified and incorporated into system models, better estimations of these populations are likely.

Additionally, improving current model parameters, particularly in the weevil modules, will be vital to refining population estimates. Maximum egg numbers per female have been recorded to vary between 125 and 1091 eggs (Del Fosse, 1977; Julien *et al.*, 1999; Julien, 2001; Jianqing *et al.*, 2002; Njoka *et al.*, 2006). Developing the current age-dependent oviposition relationship and exploring how to incorporate a maximum number of eggs oviposited per weevil would lead to more accurate weevil oviposition estimates and subsequently better estimates of egg population densities. Understanding mortality effects is also important when trying to simulate insect populations. Currently, egg survival is determined at the point of hatching but does not account for previous conditions to which each egg is exposed. Furthermore, the potential survival and development after exposure to cold (but not lethal) temperatures is not considered. Cherrill and Begon (1989) considered the development of *Chorthippus brunneus* (a grasshopper) after exposure to cold temperatures (4°C) and found that insects exposed to cold temperatures during a specific stage of egg development took longer to develop once returned to 30°C. Exposure of individuals to extreme temperatures therefore may not always result in death of an individual but may cause reductions in both survival and development when subsequently exposed to favourable conditions.

Survival rate of larvae and pupae as well as weevil longevity are likely to vary with temperature (DeLoach and Cordo, 1976; El Abjar and Bashir, 1984; McAvoy and Kok, 1999; Jianqing *et al.*, 2002; Njoka *et al.*, 2006; Oke, 2008)), but are modelled as being constant in this study. Karolewski *et al.*, (2007) showed that larval survival of *Lymantria* species depended on the temperatures to which they were exposed. Additionally, Chikwenhere (2000) showed that both larval and pupal survival of *Neochetina bruchi* declined at temperatures below and above 20°C, and that survival between larval instars was not consistent for a specific temperature. Adult longevity also varies greatly with prevailing temperatures (Njoka *et al.*, 2006; Oke, 2008). Including effective survival and longevity estimates would ensure that the effects of temperature (and potentially nutrients) are felt throughout all weevil life stages.

Realistic carrying capacities for all weevil life stages have not been explored. Wilson *et al.,* (2006) showed that as larval density of *Neochetina eichhorniae* increased in water hyacinth plants, so did larval mortality. It is also likely that adult weevil populations will be density-dependent, as are many insect populations (see Stiling, 1988). Additionally, no carrying capacity was included for egg populations in any of the models. Center (1987) observed up to 28 eggs/leaf and up to 58 eggs/plant in field populations in Florida. This gives some indication of maximum egg densities that could occur in the field, although egg densities will be highly dependent on adult fecundity and oviposition rates. Nevertheless, understanding how weevil populations may vary with temperature and density, and how these relate to realistic carrying capacities will produce more accurate weevil population estimates, and hence more accurate estimations of biological control.

Finally, to estimate water hyacinth control levels more effectively, better estimates of weevil damage need to be incorporated. Being able to quantify the relationship between weevil herbivory and *damage* to water hyacinth populations, particularly for weevil larvae, is the key to developing a model that simulates water hyacinth biological control effectively

5.2 Conclusion

Differences in temperature regimes can have extensive effects on weevil populations and subsequently the level of control exerted on water hyacinth populations. Model weevil populations remain at high densities throughout the year and produce many generations at warm sites in South Africa, but populations at cold sites are disadvantaged by winter bottlenecks, low oviposition rates and survival proportions as well as slow weevil development. Although models do not simulate water hyacinth populations accurately, they show that *Neochetina* weevils can reduce water hyacinth populations, particularly at warmer sites, and that *Neochetina* larvae are imperative to reducing plant populations. Various parameters such as age-dependent oviposition, stage-specific mortality, stage-specific carrying capacities and especially larval feeding damage need to be

further explored and combined with temperature and nutrient effects on both water hyacinth and weevil populations in order to simulate these systems accurately.

6 APPENDIX

Table A.1: List of Stage 1 (excluding temperature) and Stage 2 (temperature) water hyacinth model variables and parameters

Parameter	Units	Description	Equation	Estimate	Source
c	-	Empirical scaling constant	(Equation 5)	8.7×10^{-6}	1
K	Kg/m^2	Water hyacinth carrying capacity	(Equation 4)	70	1, 2,3
r_V	g/g/day	Intrinsic water hyacinth population growth rate	(Equation 4)	State variable	1
R	g/g/day	Relative water hyacinth population growth rate	(Equation 5)	State variable	4
T	°C	Temperature	(Equation 5)	Variable with site	4
T_{min}	°C	Minimum temperature threshold of water hyacinth	(Equation 5)	8	1
T_{max}	°C	Maximum temperature threshold of water hyacinth	(Equation 5)	40	1
V	Kg/m^2	Water hyacinth biomass density	(Equation 4)	State variable	

Source: 1) Wilson *et al.*, (2005); 2) Wilson *et al.*, (2001); 3) Wilson (2002); 4) van der Heide *et al.*, (2006)

Table A.2: List of Stage 1 (constant oviposition) insect model variables and parameters

Parameter	Units	Description	Equation	Estimate	Source
Day counter	Days	Day counter	(Equation 13)	Variable with time	
$°D$	°D	Available degree-days	(Equation 9) (Equation 10) (Equation 15)	Variable with canopy temperature	
$°D_r$	°D	Degree-day reset	(Equation 9) (Equation 11)	Variable with thermal constant	
E_d	Eggs/m^2	Egg density (daily)	(Equation 6) (Equation 7) (Equation 12) (Equation 15)	Variable with time	
E_n	Eggs/day/m^2	New eggs (Oviposition)	(Equation 6) (Equation 8) (Equation 14)	Variable with oviposition	
E_m	Eggs/day/m^2	Eggs hatching	(Equation 6) (Equation 12) (Equation 20)	Variable with temperature	
$E_{(t)}$	Eggs/m^2	Egg density (total population)	(Equation 7)	State variable	
$GDD_{(t)}$	°D	Cumulative degree-days	(Equation 9) (Equation 11)	State variable	
GDD_E	°D	Cumulative egg degree-days	(Equation 12)	125.105	Chapter 2

Parameter	Units	Description	Equation	Estimate	Source
GDD_L	°D	Cumulative larval degree-days	*(Equation 9) (Equation 22)	State variable	
GDD_P	°D	Cumulative pupal degree-days	*(Equation 9) (Equation 23)	State variable	
i	days	Initiator	(Equation 13) (Equation 14) (Equation 15)	Variable with time	
K	°D	Thermal constant	(Equation 11)	Variable with life stage	
K_E	°D	Egg thermal constant	(Equation 12)	125.104	Chapter 2
K_L	°D	Larval thermal constant	(Equation 22)	976.3	Table 3.2
K_P	°D	Pupal thermal constant	(Equation 23)	242.7	Table 3.2
L_d	Larvae/m^2	Larval density (daily)	(Equation 16)	Variable with time	
L_d	Larvae/m^2	Larval density (daily)	(Equation 18) (Equation 22)		
L_n	Larvae/day/m^2	New larvae (Hatching)	(Equation 16) (Equation 20)	Variable with temperature	
L_m	Larvae/day/m^2	Larvae maturing	(Equation 16) (Equation 21) (Equation 22)	Variable with temperature	
$L_{(t)}$	Larvae/m^2	Larval density (total population)	(Equation 18)	State variable	
O_r	Eggs/weevil/day	Oviposition rate	(Equation 8) (Equation 14)	0.75; 4	5

Parameter	Units	Description	Equation	Estimate	Source
P_d	Pupae/m^2	Pupal density (daily)	(Equation 17) (Equation 19)	Variable with time	
P_d	Pupae/m^2	Pupal density (daily)	(Equation 23)	Variable with time	
P_n	Pupae/day/m^2	New pupae (Pupation)	(Equation 17) (Equation 21)	Variable with temperature	
P_m	Pupae/day/m^2	Pupae maturing (emerging)	(Equation 17) (Equation 23)	Variable with temperature	
$P_{(t)}$	Pupae/m^2	Pupal density (total population)	(Equation 19)	State variable	
S_E	Larvae/egg	Egg survival proportion	(Equation 20)	0.96	6
S_L	Pupae.larvae^{-1}	Larval survival proportion	(Equation 21)	0.85	6
S_P	Weevil.pupae^{-1}	Pupal survival proportion	*(Equation 20)	0.95	6
T	°C	Temperature	(Equation 5)	Variable with site	5
T_c	°C	Mean daily canopy temperature	(Equation 10) (Equation 15)	Variable with site	5
T_w	°C	Mean daily water temperature	*(Equation 10) *(Equation 15)	Variable with site	5
t	°C	Lower developmental threshold	(Equation 10) (Equation 15)	Variable with life stage	
t_E	°C	Egg lower developmental threshold	*(Equation 15)	11.95	Chapter 2
t_L	°C	Larval lower developmental threshold	*(Equation 15)	5.2	Table 3.2

Parameter	Units	Description	Equation	Estimate	Source
t_P	°C	Pupal lower developmental threshold	*(Equation 15)	6.7	Table 3.2
W_d	Weevils/m^2	Weevil density (daily)	(Equation 24)	Variable with time	
W_d	Weevils/m^2	Weevil density (daily)	(Equation 25)		
W_f	Weevils/m^2	Female weevil density (daily)	(Equation 8) (Equation 14)	Variable with population	
W_i	Weevils/m^2	Initial weevil population	(Equation 24) (Equation 26)	100	
W_n	Weevils/day/m^2	New weevils (Eclosion)	*(Equation 20) (Equation 24)	Variable with temperature	
W_m	Weevils/day/m^2	Weevils dying	(Equation 24)	Variable with time	
$W_{(t)}$	Weevils/m^2	Weevil density (total population)	(Equation 25)	State variable	

Source: 5) Byrne *et al*., (2010); 6) DeLoach and Cordo, (1976)

Table A.3: List of additional/altered variables and parameters in Stage 2 (temperature-dependent) insect models

Parameter	Units	Description	Equation	Estimate	Source
O_T	°C	Oviposition temperature threshold	(Equation 27)	10	6
O_r	Eggs/weevil/day	Oviposition rate	(Equation 27)	Variable with temperature	6; Chapter 2
S_E	Larvae/egg	Egg survival proportion	(Equation 28)	variable with temperature	6
T_c	°C	Mean daily canopy temperature	(Equation 27) (Equation 28)	Variable with site	5

Source: 5) Byrne *et al.*, (2010); 6) DeLoach and Cordo, (1976);

Table A.4: List of additional/altered variables and parameters in Stage 3 (winter mortality) insect models

Parameter	Units	Description	Equation	Estimate	Source
E_w	Eggs/day/m^2	Egg winter mortality	(Equation 29)	Variable with egg population	
L_w	Larvae/day/m^2	Larval winter mortality	(Equation 30)	Variable with larval population	
T_{cmin}	°C	Minimum daily canopy temperature	(Equation 29) (Equation 30)	Variable with site	5
$W_{(t)}$	Weevils/m^2	Weevil density (total population)	(Equation 32)	State variable	
$W_{y(t)}$	Weevils/m^2	Young weevil density (total young population)	*(Equation 25) (Equation 32)	State variable	
$W_{o(t)}$	Weevils/m^2	Old weevil density (total old population)	*(Equation 25) (Equation 32)	State variable	
W_{no}	Weevils/day/m^2	New "old" adult weevils (aging)	(Equation 31)	Variable with time	
W_a	Weevils/day/m	Weevils aging	(Equation 31)	Variable with time	
W_{dy}	Weevils/m^2	Young weevils density (daily)	*(Equation 24)	Variable with time	
W_{do}	Weevils/m^2	Old weevils density (daily)	*(Equation 24)	Variable with time	

Source: 5) Byrne *et al.*, (2010);

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