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Thermal physiology components of the grasshopper *Cornops aquaticum* (Brüner, 1906) and its impact on establishment in South Africa

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Declaration

I, Giuseppe Venturi (Student number: 475587), declare that this thesis is my own, unaided work. It is submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree or examination to any other university.

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Abstract

Water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae), remains one of the worlds' worst invasive aquatic plants, causing high environmental damage and economic costs. In South Africa its damage has been reduced through the introduction of nine biological control agents, however complete control has yet to be achieved. Biological control efforts are hindered by eutrophic water bodies, but also a lack of effective biological control agents, potentially the result of climatic unsuitability between the agent and the South African climate. Despite numerous release attempts since 2011, the establishment of the water hyacinth grasshopper *Cornops aquaticum* (Orthoptera: Acrididae) has not been achieved. Sixteen years under quarantine laboratory before release may have selected for a thermally incompatible agent.

Cornops aquaticum have critical thermal minima (6.15 ± 0.12 °C) higher than other water hyacinth biological control agents, limiting their potential for establishment at many water hyacinth field sites. The grasshopper is capable of acclimation to lower critical thermal limits (5.53 ± 0.15 °C), provided adequate time for acclimation is given. Using flow-through respirometry, metabolic rates of adults were found to be significantly lower at 15 °C compared to 25 °C and 35 °C. Immature *C. aquaticum* showed significantly lower metabolic rates at 15 °C and 25 °C. *Cornops aquaticum* adults show a high mass-specific resting metabolic rate (1139.5 ± 89.97 $\mu l.hr^{-1}.g^{-1}$) at 25 °C. Outdoor experiments showed high winter mortality (> 60 %), potential problems with thermoregulation during colder conditions and an increased likelihood of adults to be found off their host plant at colder conditions. Higher temperatures (≥ 25 °C) were associated with higher feeding damage, body mass, oviposition rates, egg and instar survival and faster developmental times. Using STELLA 9.1.4, a stage-structured *C. aquaticum* population model on a temperature dependent water hyacinth model is described based on temperature data from two water hyacinth field sites.

Overall, the *C. aquaticum* population released in South Africa shows sensitivity to lower temperatures. This is probably due to selection imposed by more than 80 generations spent in temperature-controlled quarantine. Release at only the warmest sites is recommended, with release efforts focused on a single site at a time. It is suggested that the population be "renaturalised", to increase genetic diversity and thermal tolerance.

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Thesis layout

This thesis comprises seven chapters, with five research chapters. The first chapter is an introduction describing 1) the limitations of the biological control of water hyacinth, 2) the unsuccessful establishment of the biological control agent *Cornops aquaticum* in South Africa and 3) the investigation of the physiology and population modelling of *C. aquaticum.* Chapter two reports on the critical thermal limits of *C. aquaticum* adults and immatures, and the adult variation in these after acclimation and retesting. Critical thermal minima are tested for sex and body mass effects. Adult recovery rates after chill coma are also described, along with adult survival rates after exposure to sub-lethal temperatures for extended periods of time. Chapter three describes the change of the metabolic rate of adult and immature *C. aquaticum* and the effect of body mass and sex, at three temperatures. Chapter four tests the temperature preference of *C. aquaticum* adults and instars in relation to their host plant and ambient temperature. Additionally, the survival of an outdoor population was monitored over a 15-month period to investigate the impact of winter conditions at a population level. Chapter five reports on *C. aquaticum* feeding damage on water hyacinth as well as the survival and development of eggs and instars at different temperatures. Chapter six models the population dynamics of *C. aquaticum* and water hyacinth. Chapter seven is a general discussion which draws together the physiology, establishment potential as a biocontrol agent and suggestions for future release.

Problem statement

The water hyacinth grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae) was released in South Africa to control against the invasive aquatic weed water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae). After approximately 16 years in quarantine, numerous release attempts since January 2011 have been attempted with no success. Establishment of *C. aquaticum* in South Africa at sites such as Mnini Dam (30°08′56″ S 30°47′56.1″ E, Luthuli, KwaZulu-Natal) and Roodeplaat Dam (25°37′15″ S 28°22′17″ E, Pretoria, Gauteng) has been largely unsuccessful despite multiple release attempts. Climatic incompatibility is commonly proposed as an explanation for the failure of a biological control agent to become established in the recipient region. This claim will be investigated for *C. aquaticum* by several means. Determining the critical thermal minima and maxima of *C. aquaticum* may aid release procedures by identifying sites where establishment is less or more likely. Metabolic measurements over a thermal range can highlight temperatures where metabolism is depressed or maximized, emphasising the importance of temperature to the ecology of the biological control agent. Temperature monitoring at a population level and at a plant-insect level can illuminate the broad effects of temperature on populations as a whole and also how individual *C. aquaticum* grasshoppers cope with environmental conditions. The feeding rates of *C. aquaticum* at different temperatures can complement the current understanding of the nutrient effects on feeding and along with the development rate at different temperatures, assist in the future modelling of potential species efficacy at different sites and other populations. The model can more accurately predict establishment likelihood and provide a platform for future modelling of the different biological control agents and *C. aquaticum* from its native range.

Overall aims

1) Determine the critical thermal limits and metabolic rates of adults and immatures at different temperatures.

2) Determine the body temperatures and body position in relation to their host plant of adults and immatures.

3) Monitor population persistence over winter and assess developmental rates of eggs and instars.

4) Combine *Cornops aquaticum* life-history characteristics and development into a temperature model to ascertain whether establishment can occur at different water hyacinth infestation sites.

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Chapter 1

General introduction

Introduction

Plant colonization of new areas is a natural phenomenon which humans have accelerated by international movements, deliberately or neglectfully transporting plant species (Blackburn et al., 2011; Faulkner et al., 2016). Plants are generally seen as an invasive alien plant (IAP) when a non-native plant 1) is introduced into a new area that it would be unlikely to colonise naturally, 2) replaces native species through its spread and 3) causes damage to indigenous ecosystems, social or economic sectors that is deemed unacceptable (McNeely et al., 2001; Heger et al., 2013; Davidson et al., 2015). The expansion of IAPs range is facilitated by an increased transport of IAP reproductive propagules, brought about through human globalization, and by IAPs' competitive advantage over the native flora (Keane and Crawley, 2002; Leung et al., 2004; Faulkner et al., 2014, Schulz et al., 2019).

The competitive advantage for invasive plants is facilitated through habitat vulnerability (due to global climate change, overgrazing and localized nutrient enrichment), different evolutionary histories and the lack of natural enemies in the new habitat (Mack, 1989; D'Antonio and Vitousek, 1992; Mack et al., 2000; Keane and Crawley, 2002; You et al., 2014). As a result, plant invasion has been identified as a major threat to the biodiversity of natural habitats as it can influence habitat space, ecosystem services and displacement of indigenous flora (Auld and Medd, 1987; Vitousek et al., 1997; Mooney, 2005; Pyšek and Richardson, 2010). Additionally, reductions in the total water flow by IAPs has been conservatively estimated to be 1444 million m^3. yr^{-1} or 2.88 % of the South African mean annual runoff (Le Maitre et al., 2016). The implications of these reductions may be more serious in large water supply systems which, without effective management, will triple by 2045 (Le Maitre et al., 2019). These losses are significant in a water stressed country like South Africa, and where indigenous flora and fauna are particularly important (Cherry, 2005; Kapangaziwiri et al., 2018).

Estimates of water loss, biodiversity loss and loss of grazing land are approximately R6.5 billion per annum under the current state of invasion (De Lange and van Wilgen, 2010). The cost of invasions is best documented in the fynbos ecosystem of South Africa, due to its ecological value and attention from researchers (van Wilgen et al., 2001; Moran and Hoffman, 2012; van Wilgen et al., 2012). Invasions into the fynbos ecosystem have been estimated to have reduced its value by more than US $11.75 billion (Approximately R191 billion given the current exchange rate; van Wilgen et al., 2001). The impacts of IAPs in South Africa, such as threats to biodiversity and ecosystem services are assumed to be extensive and thus multiple control mechanisms have been utilized to lessen these impacts (Richardson and van Wilgen, 2004). However, most South African studies lack adequate data on the impact of IAPs, based on methods used in the International Union for Conservation of Nature (IUCN) Global Invasive Species Database (Blackburn et al., 2014; Clusella-Trullas and Garcia, 2017).

Control of alien plants

Mechanical control can successfully remove local invasions but is time-consuming, can require expensive machinery and needs to be repeated (Olckers, 1995; Charudattan et al., 1996). In addition, depending on the plant species, this method can be less effective than other control methods (Andres and Bennett, 1975). For example, mechanical control is less effective against IAP species with vegetative reproduction as the act of removal can encourage regrowth (van Valkenburg et al., 2011). Chemical control has not been utilized extensively in South Africa due to its cost and need for repeated applications (Williams and Hecky, 2005; Chamier et al., 2012).

Classical biological control programmes involve the release of a natural enemy, also called a biological control agent, on the IAPs in the country of invasion. This reapplies biotic pressure to reduce the competitive advantage of IAPs over native flora to manage the IAP population (Zachariades et al., 2017). The goal of biological control is to reduce the competitive advantage of the IAPs through the reintroduction of natural enemies, thereby reducing the IAPs' population and its economic and environmental impact to a more manageable level (Delfosse et al., 1976; Schulz et al., 2019). Classical biological control is generally considered safer than other control methods with approximately 2-13 % of released biological control agents having impacts on non-target native plants (Suckling and Sforza, 2014; Hinz et al., 2019). Thus, the risks of introduction of a natural enemy are assumed to be minimal, and justifiable when considered with the potential damage sustained without adequate control (van Wilgen et al., 2013). Nevertheless, more investigations on the indirect effects of natural enemy introduction are being implemented into pre-release testing in addition to the typical host specificity testing (Hervet et al., 2016; Schaffner et al., 2018).

Biological control success

Biological control programmes are not always successful. Approximately one third of natural enemy releases against IAPs establish successfully but, a large proportion (approximately 40 - 80 %) of these agents fail to control the IAP population completely (Hall et al., 1980; Colautti et al., 2004). Control does vary regionally, with South African biological control programmes contributing to control of 58 % of alien plants, 24 % of which are under complete control (Zachariades et al., 2017). As a result, management of certain IAPs requires utilization of multiple control measures to maintain population densities at economically acceptable population levels (Lym, 2005; Byrne et al., 2010). It must however be noted that failure and success are binary conditions describing a complex interaction whose impacts will always vary spatially and temporally.

Success of a biological control agent is judged as the significant reduction of the IAP population caused by the agent, and ideally when other control methods are not necessary (Julien and White, 1997; McConnachie et al., 2004). When biological control agents are established, they require time for their population levels to reach a level damaging enough to overtake the invasive potential of the IAP, which increases as the plant invasion grows (Reilly and Eldern, 2014). As a result, even when biological control agents are established and conditions are favourable, the reduction of the IAPs' population can take time. Usually, IAPs are not prioritised until their population has grown considerably or the negative ecological, economic, or social consequences have become apparent. Here the biological control of aquatic plants should theoretically have had an advantage as the invasion of aquatic IAPs is often more evident. However, due to the fast growth rates of aquatic IAPs, negative impacts can accumulate rapidly. In addition, precisely quantifying the influence of alien invasive aquatic plants on native ecosystems can be difficult to compare among different water bodies (Villamagna and Murphy, 2010). This is mainly owing to differences in, pre-invasion fish communities and water quality, algal concentrations, physio-chemical conditions and sampling techniques (Savino and Stein, 1982; Miranda and Hodges, 2000; Brown and Maceina, 2002). In addition, most studies do not report the ecological condition of the water body before the invasion occurred (Villamagna and Murphy, 2010).

Water hyacinth

Water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae), is native to the Amazon Basin in South America (Villamagna and Murphy, 2010; Patel, 2012). First recorded in South Africa in 1908, it was originally distributed around the world as an ornamental floating aquatic plant (Gopal, 1987; Cilliers, 1991; Villamagna and Murphy, 2010). Within South Africa, it has been viewed as the most damaging aquatic weed with regards to its widespread distribution, economic costs and environmental damage (van Wyk and van Wilgen, 2002; Coetzee et al., 2011; Hill and Coetzee, 2017; Hill et al., 2020). The types of environmental damage include increased water loss through evapotranspiration, obstruction of water bodies, increased flood events and habitat destruction (Julien et al., 1999; Villamagna and Murphy, 2010; Fraser et al., 2016). Water hyacinth has been controlled by mechanical removal, herbicides and the introduction of biological control agents. Chemical control has the potential to have adverse effects on water quality, thus affecting fish, livestock and humans (De Groote et al., 2003). Due to the high cost of mechanical and chemical control of water hyacinth, especially for developing countries, and given control is often fleeting, a more economically sustainable long-term solution is necessary (Cilliers, 1991; Charudattan et al., 1996; Williams and Hecky, 2005; Chamier et al., 2012; Hill and Coetzee, 2017).

The water hyacinth biological control programme in South Africa includes two weevils, *Neochetina eichhorniae* (Coleoptera: Curculionidae) and *Neochetina bruchi* (Coleoptera: Curculionidae), which were respectively introduced in 1974 (with reintroductions in 1977 and 1985) and 1989 (Cilliers, 1991). Two other insect species, a moth, *Niphograpta albiguttalis* (Lepidoptera: Pyralidae) formerly *Sameodes albiguttalis* (Lepidoptera: Crambidae), and a leaf sucking mirid, *Eccritotarsus catarinensis* (Hemiptera: Miridae), were introduced in 1990 and 1996, respectively (Julien, 2001). Recent morphological and molecular evidence have found *E. catarinensis* in South Africa, collected from populations in Peru and Brazil, contained a cryptic species *Eccritotarsus eichhorniae* (Hemiptera: Miridae; Paterson et al., 2016; Paterson et al., 2019). A pathogenic fungus, *Cercospora piaropi* (Mycosphaerellales: Mycosphaerellaceae) and a mite, *Orthogalumna terebrantis* (Acarina: Galumnidae) were discovered on the weed in 1987 and 1990 respectively and were not intentionally introduced. The water hyacinth grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae) was released in South Africa in January 2011 (Bownes and King, 2010; Bownes et al., 2011). The plant hopper, *Megamelus scutellaris* (Hemiptera: Delphacidae) has been established in Florida and other areas of the USA and appears to be damaging. Due to its recent establishment (2013) at some sites in South Africa, its full efficacy has not yet been evaluated but it does appear to be promising in colder regions (Heard et al., 2014; Tipping et al., 2014; Hill and Coetzee, 2017). Despite biological control initiatives since 1974, variation in the degree of control has been noted at different sites within South Africa (Coetzee et al., 2011). In spite of the success of biological control agents with other floating aquatic weeds and the release of multiple potentially damaging biological control agents, complete control of water hyacinth has yet to be attained (Hill and Olckers, 2001; Pratt et al., 2013; Hill and Coetzee, 2017; Hill et al., 2020). Although there has not been complete control of water hyacinth, there have been significant reductions in the economic and social impacts of the water hyacinth through the use of biological control agents (Hill and Coetzee, 2017). The irregular control has been largely attributed to climatic unsuitability between the water hyacinth and its biological control agents, and to South African water bodies containing high levels of nutrients as a result of eutrophication (Hill et al., 1999; Jadhav et al., 2007). Hydrology and management with herbicides have also been used to explain the irregular spatial and temporal control (Cilliers, 1991; Hill and Olckers, 2001). In response to inadequate control, three primary approaches have been proposed.

The first is integrated management using chemical, mechanical and biological control with the intention to maximise synergistic effects while lessening conflicting outcomes (Byrne et al., 2010). Secondly, host-specificity testing for additional biological control agents should be performed to increase overall natural enemy damage (Bownes, 2009; Franceschini et al., 2010; Coetzee et al., 2011). Thirdly, a better understanding of the interaction of IAPs and their natural enemies can improve biological control agent selection and release protocols (Franceschini et al., 2010; Coetzee et al., 2011). Thus, in order to improve water hyacinth control, additional biological control agents should be obtained and investigations to improve understanding of the current suite of biological control agents should be performed (Perkins, 1974; Cock et al., 2010).

Cornops aquaticum

Cornops aquaticum is a semi-aquatic neotropical grasshopper with a wide distribution ranging from Mexico, through South America to central Argentina and Uruguay (Adis et al., 2007; Figure 1.1). Observations from the Argentine floodplains in the Middle Paraná River indicate that the first three instar stages of *C. aquaticum* are monophagous on water hyacinth, while the fourth and fifth instar stages and adults show polyphagy on several other macrophytes, mainly *Paspalum repens* (Poales: Gramineae) and *Ludwigia peploides* (Myrtales: Onagraceae) (Capello et al., 2011). Adults are winged and are strong flyers and swimmers (Silva et al., 2014). In the native range, *C. aquaticum* exhibit changes from green to brown during the wet and dry seasons, however, this change was not noted consistently in the South Africa laboratory specimens. The grasshoppers predominately feed on the third, fourth and fifth leaves of water hyacinth (Penfound and Earl, 1948; Bownes, 2009).

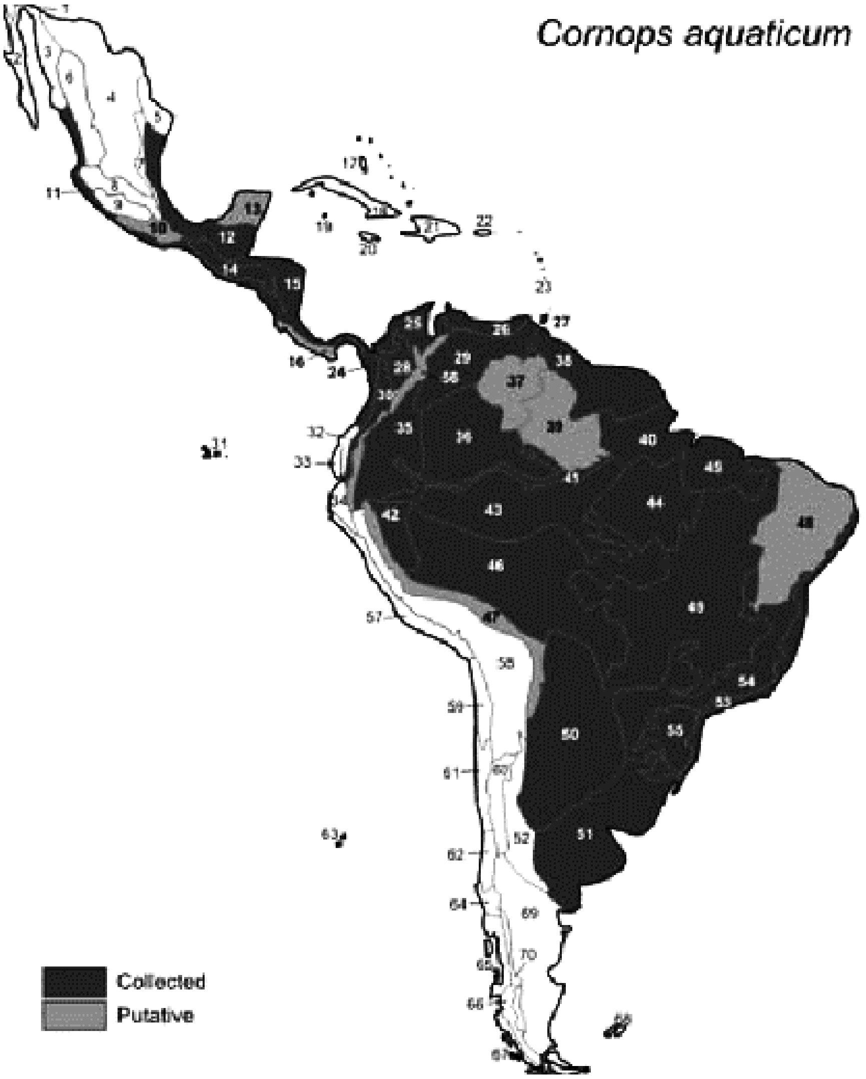


Figure 1.1. The distribution of Cornops aquaticum in South America. Collected and putative highlight the actual and presumed area of occurrences (Adis et al., 2007, pp 13) based on biogeographic provinces (adapted from Morrone, 2006).

Cornops aquaticum is considered a worthwhile biological control agent based on the considerable damage it causes to water hyacinth in its native range (Franceschini et al., 2013). Based on observations of the growth capacity of water hyacinth in Mexico, even this highly damaging grasshopper is unlikely to be able to successfully control the plant on its own in South Africa (Adis and Junk, 2003; Jimenez and Balandra, 2007). However, the capacity of *C. aquaticum* to reduce water hyacinth competitive ability through leaf biomass removal, suggested it would be a good addition to the current biological agents in South Africa used against water hyacinth (Bownes et al., 2010a; Bownes et al., 2010b). Increased feeding by natural enemies can reduce biomass or competitive ability of an IAP until a new population equilibrium is reached (Center et al., 2005). As such, studies in South Africa have shown that *C. aquaticum* can reduce the competitive ability of water hyacinth against water lettuce, *Pistia stratiotes* (Alismatales: Araceae) (Bownes et al., 2010b).

The South African population of *C. aquaticum* was originally collected on the first of October 1995 in Brazil, Amazonas, Manaus, Isla Terra Nova (3°05’05” S 59°44’07” W; Hill and Oberholzer, 2000; Figure 1.2). The South African *C. aquaticum* population after 13 years in laboratory conditions were found to be smaller and have a genetic diversity more similar to isolated populations in South America (Adis et al., 2008). The South African *C. aquaticum* population have now been bred in laboratory conditions for close to 25 years. More than twenty releases of over 8000 *C. aquaticum* individuals have taken place in total since January 2011 at ten different sites in South Africa (Anthony King, pers. comm.; Table 1.1). Despite several release attempts at these sites, establishment has yet to be confirmed. Early release attempts of *C. aquaticum* in 2011 were performed at four different sites with 300 individuals released per site (Bownes et al., 2011). However, recent adjustments in release protocols where greater numbers of adults were released did lead to more promising population persistence at Mnini Dam (Anthony King, pers. comm.). Regardless, understanding factors responsible for the failure or limited establishment of agents may assist in guiding release strategies for *C. aquaticum* and other agents (Scott et al., 2016).

Figure 1.2. Timeline from the collection of *Cornops aquaticum* individuals in 1995, the release of *C. aquaticum* in South Africa in 2011 until present day.

Table 1.1. Water hyacinth infested field sites at which Cornops aquaticum has been released.

Site name	Province	Latitude	Longitude
Golden Citrus	Mpumalanga	25°26'08.2" S	31°39'21.6" E
Misty Hills	Gauteng	26°02'05.2" S	27°51'17.2" E
Mnini Dam	KwaZulu-Natal	30°08'35.6" S	30°48'30.4" E
Roodeplaat	Gauteng	25°36'12.2" S	28°21'18.5" E
Clairwood Quarry	KwaZulu-Natal	29°54'25.1" S	30°57'17.0" E
Stellenbosch	Western Cape	33°49'01.3" S	18°56'49.3" E
Dudley Pringle	KwaZulu-Natal	29°32'01.7" S	31°08'06.4" E
Diep River	Western Cape	33°51'02.2" S	18°30'24.4" E
Hansmoeskraal	Western Cape	34°01'55.0" S	22°27'01.0" E
Matubatuba	KwaZulu-Natal	28°25'02.6" S	32°10'59.2" E

Factors such as parasitism and predation are known to negatively affect *C. aquaticum* populations in their native range (Silveira-Guido and Perkins, 1975; Ferreira and Vasconcellos-Neto, 2001). *Cornops aquaticum* is reported to be susceptible to a specialist egg predator, the weevil, *Ludovix fasciatus* (Coleoptera: Curculionidae) (Zwolfer and Bennett, 1969; Silveira-Guido and Perkins, 1975). However, this species does not occur in South Africa. Predators can have pronounced effects on prey by reducing overall activity, increasing vigilance behaviour, modifying habitat selection, altering prey movements and affecting pathogen prevalence (Nelson et al., 2004; Ahnesjö and Forsman, 2006; DeBano, 2006; Ji et al., 2008; Thaler et al., 2012; Long and Finke, 2015). As a result, generalist predators such as spiders, frogs, fish and birds may pose a threat to founder populations of *C. aquaticum*.

Climatic incompatibility is commonly thought to be a key abiotic factor constraining biological control agent establishment and/or efficacy (Andow et al., 1997; Hill and Olckers, 2001; Day and Zalucki, 2009; Zachariades et al., 2009). Recent ecogeographical habitat suitability modelling has shown that water hyacinth in South Africa has gone through a niche shift to occupy habitats at colder temperatures than those in their native range (Smit et al., 2014). Given this niche shift, a better understanding of the thermal biology of biological control agents of water hyacinth is vital to improve agent establishment and efficacy as temperature governs development, behaviour response to predation and even susceptibility to disease (Barton, 2011; Kistner and Belovsky, 2013; Marvin et al., 2016). Investigations of the effect of temperature on the aforementioned factors governing populations, may highlight the potential trade-offs occurring between different factors (Mangel and Stamps, 2001). For example, grasshoppers are likely to attempt to maintain certain body temperatures potentially to optimize developmental rates regardless of predation risk (Chapman and Joern, 1990; Hertz et al., 1993). Therefore, *C. aquaticum* may be able to compensate for lower environmental temperatures by basking but this may increase their overall predation risk (Pitt, 1999; Barton 2010).

Thermal Biology

Thermal biology can be assessed in different ways, which highlight different temperature limitations and performance measures of an organism (Chown and Nicolson, 2004). These range from investigations of the temperatures a species can physiologically withstand, the thermal sensitivity of metabolic rates, preferred temperatures of the species and investigations of life-history characteristics (LHCs). These life history characteristics include feeding, survival and overall development, which can be strongly influenced by different temperature regimes.

Upper and lower lethal limits are a means for evaluating survival where after exposure to a certain temperature for an extended period mortality is determined (Kimura, 2004; Andersen et al., 2015). The critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) are defined as the temperatures at which the organism begins to show uncoordinated movement and muscular spasms (Terblanche et al., 2007; Rezende et al., 2011). These critical thermal limits can be understood as a behavioural range, signifying the points at which the organism can no longer respond to stimuli from its environment and this can be estimated through laboratory investigations (Dixon et al., 2009). Temperature induced torpor can be defined as a state where physiological activity is largely halted as a result of either temperature extremes (minimum and maximum), referred to as chill coma in the case of CT_{min} (Hazell and Bale, 2011). At lower temperatures insect activity is reduced to the point where no movement occurs and oxidative stress is accumulated (Hazell et al., 2010; Lalouette et al., 2011). As the temperatures increase insects experience impaired coordination, reduced membrane integrity and increasing difficulty in maintaining metabolism (Neven, 2000; González-Tokman, 2020).

The laboratory determined temperature limits are generally an underestimation of temperatures experienced in the field due to the behaviour response to seek microhabitat thermal refuges (Terblanche et al., 2007). Static or dynamic methods are generally employed to test thermal stress at high and low temperatures (Lutterschmidt and Hutchison, 1997; Hoffmann et al., 2003). Static methods assess survival at a certain time after a temperature stress is experienced (Krebs and Loeschcke, 1995a, 1995b), while dynamic methods test specimens at a constant temperature rate for a certain time period and generally record at what temperature locomotory function is halted (Terblanche et al., 2007). Both provide insight into the manner in which the thermal stress is experienced (Lutterschmidt and Hutchison, 1997).

Two important factors affecting the perceived thermal tolerance range of an organism are the duration of exposure to unfavourable conditions and the time assigned for recovery before results are collected (Baust and Rojas, 1985; Bale, 1993). A moderate thermal stress, if experienced over a long duration, can potentially result in thermal stress similar to the thermal stress of short durations at lethal temperatures (Bale, 1996; Nedvěd et al., 1998). Thermal stress, specifically cold stress, can be problematic for species from warm regions, which can accumulate cold-induced injuries at moderate temperatures (Sinclair et al., 2015; Overgaard and MacMillan, 2017). Therefore, it is critical that laboratory estimations attempt to match the environmental conditions. As important as the thermal range of the organism is, slow recovery from torpor can be problematic too as it extends the time at which the organism is unable to react to predators. In addition, if organisms have the ability to improve their thermal range, potentially through acclimation, it can reduce their sensitivity to extreme temperatures and allow them more time to feed, avoid predation and find mates. Acclimation can be defined as exposing organisms to mildly stressful temperatures for a certain period, usually over days to weeks (Bowler, 2005; Loeschcke and Sørensen, 2005).

How metabolic rate increases with higher temperatures is also an important measure to help understand a species' persistence beyond the binary survival test of temperature extremes. Metabolic rates are intricately connected with body temperature, and together shape LHCs such as development rates, feeding, longevity and fecundity (Slansky and Scriber, 1985; Garrad et al., 2016). In addition, habitat temperature variability is known to diminish the sensitivity of metabolism to temperature changes, which may be important for a species that lives on a plant that acts as thermal buffer (Williams et al., 2012). Higher resting metabolic rates at various temperatures are associated with high resource abundance which tends to favour highly active competitive species (Auer et al., 2015; Mathot et al., 2015; Reade et al., 2019). Higher metabolic rates are associated with faster growth rates but require high daily energy expenditures and may result in higher predation risk (Cano and Nicieza, 2006; Biro and Stamps, 2010; Krams et al., 2013). The understanding of a species' metabolic changes with temperature can highlight temperature regions in which metabolic rate will be reduced and thus how the LHCs may be affected. Metabolic rates also highlight important sex and body mass related differences in response to temperature which may hinder establishment. Metabolic rate and gas exchange patterns can change depending on different factors, such as temperature, activity and water loss (Quinlan and Hadley, 1993; Contreras and Bradley, 2010). Three gas exchange patterns are noted in insects: continuous, cyclic and discontinuous (Chown, 2011). These patterns can provide information on the interactions between the environment and the organisms' physiology (Chown, 2011; Huang et al., 2015).

Phenological events, or the timing of certain life cycle stages, are very important in the selection of a good biological control agent as the sensitivity of phenology to temperatures and other climatic conditions is well documented (Thackeray et al., 2016; Kharouba et al., 2018). The change in the rate of development under different temperatures as well as the reproductive potential and mortality are key aspects of successful biological control agents (Jian et al., 2018). The impact of temperature on insect development has been investigated based on the assumption that a particular species needs to accumulate a specific number of thermal units called degree-days (°D) over time in order to complete development (Roltsch et al., 1999; Damos and Savopoulou-Soultani, 2012). This stems from the law of total effective temperatures, which provides a means for the calculation of the physiological time for a particular developmental stage to be completed (Damos and Savopoulou-Soultani, 2012).

The developmental rate of insects generally increases with temperature until an optimal range is reached (Sharpe and DeMichele, 1977; Sharpe et al., 1977; Hartley and Lester, 2003). This relationship can be used to estimate insect phenology, or how the timing of season activities can change at different temperatures and how these may change with climate change (Zuo et al., 2012; Cohen et al., 2018; Despland, 2018). Multiple models have been created to approximate this relationship. The oldest and perhaps the simplest of which is the linear intercept method proposed by Campbell et al. (1974). The line intercept method uses the formula:

$$Y = a + bT$$

(Equation 1)

Where Y is the developmental rate (1/days) and T is the ambient temperature (^oC) that the insect is reared at. Due to its simplistic construction, its accuracy in developmental threshold (t) and degree-day requirements (K) estimates has been questioned (Ikemoto and Takai, 2000). Developmental threshold (t) is the temperature at which development is halted, while the degree-day requirement is the duration required for development. Ikemoto and Takai (2000) put forward an alternative, the reduced major axis regression method, represented by the equation:

$$DT= K +tD$$

(Equation 2)

Where D is the developmental duration and DT is the product of the developmental time and the ambient temperature at which the insects are reared (^oC). This method is thought to reduce errors in t and K estimates and thus will be preferentially used in this study (Ikemoto and Takai, 2000).

Laboratory studies of *C. aquaticum* eggs have shown that between 25 and 30 days are required for hatching (Hill and Oberholzer, 2000; Oberholzer and Hill, 2001). Bownes (2009) showed *C. aquaticum* instars require a mean of 39 ± 2.45 days to develop into adults at high nutrient conditions (nitrates 7.6 mg/L and phosphates 1.37 mg/L) but this can increase to 41.9 ± 1.89 days if the nutrient conditions are low (nitrates 0.034 mg/L and phosphates 0.024 mg/L). *Cornops aquaticum* typically goes through five to seven instars depending on the photoperiod and climatic conditions in different areas (Adis et al., 2004; Brede et al., 2007; Capello et al., 2007). This variation is also found in South Africa, where five instars were noted by Bownes (2009) but six to seven instars were noted by Oberholzer and Hill (2001).

The reproductive potential of a species can be incredibly important in the selection of good biological control agents (Pilkington and Hoddle, 2006; Aboubakar Souna et al., 2017). The fecundity of a species relies on the number of eggs a female can produce in her life-span which is a function of her life-span and her oviposition rate (number of eggs oviposited by one female per day), which can change at different temperatures (Zilahi-Balogh et al., 2003; Aboubakar Souna et al., 2017). Grasshopper egg hatching has been shown to generally vary from 50 - 100 % (Chapman and Joern, 1990; Stauffer et al., 2011). However, high and low temperatures can dramatically reduce this (Pickford, 1972; Chapman and Joern, 1990; Dysart, 1991; Wu et al., 2013). The survival of a species relies on how each stage survives to the next stage. This is influenced by the developmental rate as well as individuals lost to predation, particularly at earlier instars, or to climatic events (Zilahi-Balogh et al., 2003; Hatherly et al., 2005; Pilkington and Hoddle, 2006). *Cornops aquaticum* females lay a concealed egg packet containing between 30 and 70 eggs, with a frothy egg foam plug, into the youngest petiole of the water hyacinth plant (Hill and Oberholzer, 2000; Oberholzer and Hill, 2001). Females first brought into quarantine showed high variation in the number of offspring they produced throughout their lifespan. Females oviposited between one and seven egg packets producing between 50 to 560 individuals (Hill and Oberholzer, 2000; Oberholzer and Hill, 2001). The lifespan of *C. aquaticum* adults has been observed to range from 50 -120 days in different regions (Oberholzer and Hill, 2001; Adis and Junk, 2003; Vieira and Santos, 2003). Females tend to live longer with an average lifespan of 120 days compared to 90 days in males (Adis and Junk, 2003).

In most grasshoppers, instar mortality appears to be highest at early developmental stages but can vary considerably due to weather conditions, disease and predation (Oedekoven and Joern, 1998; Branson, 2014; Kistner and Belovsky, 2014). Danner and Joern (2003) measured laboratory survival to the adult stage of the common rangeland grasshopper, *Ageneotettix deorum* (Orthoptera: Acrididae), to be approximately 19.4 % and 36.5 % with and without predation respectively. The instar laboratory survival to adulthood of the edible grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae), was 38.1 % (Malinga et al., 2018), while the average instar laboratory survival to adulthood in pygmy grasshoppers was 85 %, but ranged from 50 - 100 % (Forsman, 2011). Bownes (2009) had laboratory survival to adulthood of approximately 40 % and 60 % for female *C. aquaticum* reared on plants grown at low and high nutrient solutions respectively while males had survival to adulthood of approximately 65 % and 38 % (Bownes 2009).

The level of plant damage at different sites is of paramount importance to biological control efforts of IAPs. Overall insect damage to the host plant is affected by individual damage by the natural enemy, the ability of the host plant to compensate and the population density of the natural enemy (Maron and Crone, 2006; Stephens et al., 2013). For example, the population density of *Salvinia molesta* (Salviniales: Salviniaceae) is strongly influenced by the number of adult *Cyrtobagous salviniae* (Coleoptera: Curculionidae) present but also the damage caused by each individual, which is strongly influenced by overall temperature (Mukherjee et al., 2017). Efforts have been made to understand the reduction in the efficacy of biological control agents on IAPs in high nutrient water bodies (Coetzee et al., 2007). The interaction is complex, in that high nutrient bodies assist with natural enemy establishment but generally reduce the overall efficacy of the biological control agent, most likely due to plants being able to better compensate for biomass removal (Heard and Winterton, 2000; Wilson et al., 2006; Coetzee et al., 2007; Mukarugwiro et al., 2018).

The plant compensation can be overcome if the natural enemy population is large enough. For example, Bownes et al. (2010a) suggested that *C. aquaticum* will suppress water hyacinth growth in higher nutrient conditions and reduce water hyacinth biomass when grasshopper densities were greater than one grasshopper per plant. However, in its native range the ratio of grasshopper to plant is usually 1:1, emphasizing the potential problem in control attempts of freshwater bodies experiencing eutrophic conditions (Silveiro-Guido and Perkins, 1975). It should be noted that *C. aquaticum* abundance in the native range shows high variation across seasons, years and across different vegetation patches (Franceschini et al., 2013). In addition, natural enemy consumption rates can also fluctuate depending on ambient temperature (DeLoach and Cordo, 1976; Ferro et al., 1985; Lactin and Johnson, 1995; Chikwenhere, 2000). It is important therefore to quantify the rates of feeding damage and responses of *C. aquaticum* in different temperature conditions.

Modelling

Models are a representation of reality, used to facilitate the understanding of species and factors affecting their population. General models have been developed to model single species and interacting populations (Kot, 2001). Models of single species include logistic and exponential growth models, harvest models and discrete and delay models (Kot, 2001). The function of these models can vary, with attempts to eradicate invasive species or maximum harvest that can be achieved over multiple years (Maron and Crone, 2006; Baker et al., 2017; Ayllón et al., 2019). Interacting species models attempt to represent classical predator-prey interactions as well as competitive interactions between two or more species (Kot, 2001). However, all models can be separated into two model scenarios; complex models which are accurate but require more information and simple models which are less accurate but easier to create (Priesmann et al., 2019). Both types of models play a pivotal role in the prediction and simulation of complex systems, increasing the understanding of the system in question (Freckleton and Stephens, 2009). As a result, a variety of models exist, such as niche and mathematical models, and can usually be tailored to a particular system (Ogden et al., 2005).

Biological control has most commonly used niche models as they allow matching of meteorological values between the area of invasion and the native distribution (Zalucki and van Klinken, 2006). These models, referred to as ‘climatic envelopes’ or species distribution modelling, provide information on the theoretical or potential physiological distribution globally, as well as the possible seasonal fluctuations of the subject plants or insects (Beaumont et al., 2009; Elith and Leathwick, 2009). As a result, climatic matching can be particularly useful to identify suitable areas to locate biological control candidates, sites at which they can be released and their potential distribution (Hoelmer and Kirk, 2005; Srivastava et al., 2019). The main limitations of these types of models are that microhabitats are often not taken into consideration and that ecological variables such as population level, developmental rates or other LHCs are often not considered (Aurambout et al., 2009; Morin and Thuiller, 2009; Ellis, 2018).

Mathematical modelling of population dynamics has been particularly useful in the ecological understanding of invasion and control protocols, such as non-target impacts and the ability of a biological control agent to control their respective weed under different environmental factors (Wilson et al., 2005; Kriticos et al., 2009; Anguelov et al., 2017). Mathematical based models simulating biological processes require specific data, but can allow a more comprehensive understanding of the system than ones lacking information about the biological system (Plouvier and Wajnberg, 2018). The population levels of the IAP and its natural enemy, coupled with their respective LHCs, can assist in understanding their interaction and thus contribute to the control of the IAP (Mills and Kean, 2010; Plouvier and Wajnberg, 2018).

The incorporation of species-specific LHCs into a model can allow a greater level of reliability that the system has been accurately described and that predictions made are more likely to be accurate (Anguelov et al., 2017). The modelling of the interaction between the IAP and its natural enemy along with its LHCs must also be closely tailored to the actual environmental conditions that the organism will experience. Once a rudimentary model of the system has been created, testing how sensitive the population abundance and dynamics are to changes in LHCs can further improve understanding of this interaction (Fatichi et al., 2018; Delattre et al., 2019). However, as models are simplifications of essential systems, only key LHCs should be utilized. Modelling of biological control processes require modelling of both the IAP and the biological control agents' populations over time and quantifying how the two interact. This requires the populations to be described, either by their density, biomass or the total number of individuals in a particular area.

Populations are not static and vary spatially and temporally. Various processes, most broadly described as biotic and abiotic factors, dictate the size of a population through time, also known as population dynamics. As a result, multiple types of models have been used to represent the growth of populations over time, the most common of which is the exponential and logistic type models (Otto and Day, 2011). Modelling insect development relies on the assumption that the relationship between development and temperature are linearly correlated above a certain minimum temperature required to initiate development (Campbell et al., 1974; Snyder et al., 1999; Damos and Savopoulou-Soultani, 2012). As temperatures increase, developmental rates increase proportional to those increasing temperatures until an upper threshold limit is reached which affects enzyme activity thereby slowing developmental time (Wagner et al., 1984; Rebaudo et al., 2018).

Understanding the timing of development, at different temperature regimes, is essential to predict the emergence time and sensitive life stages (Moore and Remais, 2014). This together with how the population overwinters can be key aspects to determining population dynamics over time (Prado et al., 2015). For example, in the Madeira mealybug, *Phenacoccus madeirensis*

(Hemiptera: Pseudococcidae), adults were well protected from both chemical and biological control due to their thick waxy secretions and long lifespan and thus control was difficult and not very effective (Vennila et al., 2010; Saddiq et al., 2014). Better control could be facilitated through the selection of appropriate temperatures regimes, better natural enemies and particular insecticides to target the most susceptible instar stages (Chong et al., 2003; Saddiq et al., 2015; Tong et al., 2019). Generation numbers can also be helpful in predicting invasion success, predicting establishment and prioritizing important areas for control (McClay and Hughes, 1995; Herms, 2004; Pilkington and Hoddle, 2006).

Despite increasing popularity of non-linear model and their success, linear models such as the degree-day accumulation model provides a good starting point to the development of more complex models. For example, Prout and McChesnay (1985) produced a simple model base where *Drosophila melanogaster* (Diptera: Drosophilidae) adults and larvae were isolated in discrete time steps to note if intrapopulation competition affected adult fertility. This simple model has been improved and modified by different authors to better understand the interaction of different stages on one another and the implications to pest-control methods such as the sterile insect techniques (Lai and Bradbury, 1998; Tung et al., 2017). In addition, many simplistic degree-day models have been used to successfully predict the life history of many insects (Purcell and Welter, 1990; Moore et al., 2012).

Thus, thermal biology is a key aspect to understand the lack of establishment of biological control agents. Components investigated in this study are; behavioural limits and whether they can be altered, metabolic rates over a range of temperatures and the effect of temperature on LHCs such as fecundity, feeding rates, survival and development. In addition, a mathematical model is described to ascertain if *C. aquaticum* can develop at different water hyacinth sites and what level of control they may be able to provide.

Chapter 2

Behavioural thermal limits, recovery and thermal acclimation in *Cornops aquaticum* adults

Introduction

The ecological niche of a species is strongly influenced by its tolerance and performance under different environmental variables. An understanding of a species' niche is of particular importance when translocating natural enemies, usually insects, of invasive alien plants (IAPs) into the area of invasion. The stochastic nature of environmental variables and their importance of to insectspecies can lead to difficulties in the establishment and success of biological control agents (Broennimann et al., 2007). Climatic incompatibility is thought to account for more than 40 % of known reduced impacts of biological control agents and problems of establishment at release sites (Colautti et al., 2004; Day and Zalucki, 2009; Zachariades et al., 2009). The native distribution of the biological control agent and the invasive plant are expected to have similar climatic ranges (Sheppard, 2003; Hinz and Schwarzländer, 2004). However, invasive alien plant species can represent a narrow proportion of their native population (Smit et al., 2014; Gitonga et al., 2015) that have possibly undergone a niche shift either through rapid adaptation or via increased phenotypic plasticity (Broennimann et al., 2007; Roderick and Navajas, 2008). Phenotypic plasticity is defined here as the ability of an individual to modify its morphology/physiology when subjected to different environmental conditions. Therefore, the success of biological control may be aided by introducing agents with the broadest physiological tolerances or the capability to adjust to the new niche of the invasive plant species (Zepeda-Paulo et al., 2015). The niche of a species involves a suite of environmental variables, of which only the most important variables must be selected for experimentation (Guisan and Thuiller, 2005; Braunisch et al., 2013). Temperature is often chosen as it affects a number of important life-history characteristics in insects (Gillooly et al., 2001; Grazer and Martin, 2012; Régnière et al., 2012).

Temperature studies usually involve determining the thermal boundaries an insect can tolerate and the performance within those boundaries to establish an insects' optimal thermal conditions and temperatures where performance may be poor. These types of studies have received new attention in light of global climate change (Sørensen et al., 2016; Hoffman and Sgrò, 2018). These critical thermal limits provide key information about whether species can occur in particular regions. Certain characteristics of the species should be considered with the critical thermal limits to better understand their thermal biology. For example, different thermal preferences may occur between sexes, and body mass can affect critical thermal limits due to the thermal inertia (Forsman, 2000; Neargarder et al., 2003; Ahnesjö and Forsman, 2006). In the pygmy grasshopper, *Tetrix subulata* (Orthoptera: Tetrigidae), females preferred to maintain higher body temperatures than males, which could signify differences in the thermal range, recovery after chill coma and acclimation between sexes (Forsman, 2000). These estimates can also be altered as a result of experimental manipulation of the thermal stress and the rate of temperature change and selecting for different thermal traits (Chown and Nicolson, 2004; Terblanche et al., 2007; Alford et al., 2016).

Slower heating and cooling rates often lead to higher critical thermal maxima (CT_{max}) and lower critical thermal minima (CT_{min}) (Terblanche et al., 2007; Chown et al., 2009; Terblanche et al., 2011). The CT_{max} and CT_{min} are defined as the upper and lower temperature at which an organism begins to show uncoordinated movement and muscular spasms (Terblanche et al., 2007; Rezende et al., 2011). For instance, Powell and Bale (2006) showed that aphids cooled at 0.1 $^{o}C.min^{-1}$ went into torpor (chill coma) at lower temperatures than those cooled at 0.5 $^{o}C.min^{-1}$ and 1 $^{o}C.min^{-1}$. In contrast, studies with *Glossina pallidipes* (Diptera: Glossinidae) and *Drosophila melanogaster* (Diptera: Drosophilidae) have shown that slow rates of cooling can lead to CT_{min} elevation, possibly due to the extended time at these stressful low temperatures (Overgaard et al., 2006; Terblanche et al., 2007).

Ideally, to examine an organism's performance over its entire thermal range, critical thermal temperatures must be estimated, along with how quickly, and to what degree, the organism can acclimate itself to a new thermal environment (Terblanche et al., 2007). For example, if an organism acclimates to a thermal stress quickly, then that organism may be able to survive and increase its thermal tolerance, despite an earlier test revealing a narrow thermal range. The ability of an organism to modify its thermal range when necessary may thus be of equal importance to the critical thermal limits.

Experiments of physiological modification such as rapid heat/cold hardening and acclimation have shown that the thermal tolerance of species can change in a short space of time (Sørensen et al., 2016). These studies have also highlighted the implication to field performance, where cold acclimation of biological control agents, improved their efficacy at lower temperatures (Sørensen et al., 2016; Terblanche, 2014). Rapid heat/cold hardening experiments apply temperature stress as a severe heat/cold shock for a very brief time period. Acclimation experiments deliver a more moderate temperature stress for a longer period of time (Loeschcke and Sørensen, 2005; Sinclair and Roberts, 2005; Wang et al., 2011). Despite the differences in the way the thermal stress is applied, many have argued that species' responses to thermal stresses are similar and heat/cold hardening may just result in a more intense adaptation response (Bowler, 2005; Loeschcke and Sørensen, 2005; Sinclair and Roberts, 2005).

Cold hardening refers to the short-term adaptation to extreme low temperatures following brief exposure to a sub-lethal temperature (Bowler, 2005). Cold and heat shock denotes a short-term response to a brief duration at sub-lethal temperatures, usually accompanied by the production of short acting protective molecules such as glycerol and heat shock proteins (Bowler, 2005). The sub-lethal temperatures can also cause thermal stress, which if applied for a long enough duration can accumulate, cause loss of condition and lead to death (Bale, 1996; Kingsolver and Woods, 2016; Lubawy et al., 2019). Another important characteristic when investigating how organisms interact with temperature is their recovery from near lethal temperatures (David et al., 1998). The ability to recover, particularly from chill coma, allows organisms to return to their normal behaviour more quickly and their time in a vulnerable state can be reduced. Thus, the perceived thermal tolerance range of an organism is affected by the duration of exposure and the rate of temperature change (Chown and Nicolson, 2004; Rohr et al., 2017). Thereafter, recovery and acclimation become important factors in order to assess how well an organism will do in a particular environment.

Investigations of the thermal range of biological control agents must therefore attempt to match the manner in which the thermal stress is experienced in the field and the laboratory. The water hyacinth control agents *Neochetina eichorniae* (Coleoptera: Curculionidae), *Eccritotarsus catarinensis* (Hemiptera: Miridae) and *Orthogalumna terebrantis* (Acarina: Galumnidae) have had their thermal range investigated to reveal possible sensitivities to colder water hyacinth sites (Coetzee et al., 2007; Byrne et al., 2010; Marlin, 2011). An important addition when considering a species inhabiting a broad ecological area in the native range (Adis et al., 2007), such as *Cornops aquaticum* (Orthoptera: Acrididae), is that acclimation may be an important factor in extending their thermal range. The key aim of this chapter is to determine the thermal range and recovery time of *C. aquaticum* reared in South Africa. Once the basic thermal range is established, the objectives become, 1) to what degree acclimation could modify the thermal range of *C. aquaticum* and recovery time from chill coma and 2) if acclimation is possible, what is the survival rate of *C. aquaticum* individuals after prolonged exposure at low temperatures before acclimation can potentially assist in the extension of their behavioural range?

Materials and methods

Study species

Cornops aquaticum individuals for these experiments were supplied by the Weeds Research Division of the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI) in Rietondale, Pretoria. Grasshoppers were kept at the University of the Witwatersrand greenhouse, Johannesburg, South Africa (26°11’26.9” S 28°1’52.8” E) from December 2014 in a 93 cm x 111 cm x 45 cm container with water hyacinth plants and covered with a fine mesh. In October 2015 the culture was moved to the University of the Witwatersrand Insectary and Quarantine Facility and kept in 45 L buckets covered with a fine mesh. Temperature and humidity inside the insectary were recorded with Thermochron® iButton® devices (DS1921G) and a digital thermos-hydrometer (ZEAL). The temperature ranged between 15 °C and 35 °C, while the humidity was 50 ± 10 %. Experiments were conducted within laboratory facilities at the University of the Witwatersrand.

Experimental design and protocol

Critical thermal limits

A Labcon low temperature programmable water bath (LTB 12/30) with a Labcon circulator (CPE50) was used to test *C. aquaticum* individuals’ upper and lower thermal limits (CT_{max} and CT_{min}). Ten males and ten females (referred to as the “stock”) were removed from the main *C. aquaticum* population and weighed using a semi-micro Shimadzu balance (Libror AEG-45SM; accuracy ± 0.00001 g) and placed in separate empty glass vials (20 ml) sealed with moist cotton wool. Sex was ascertained through the identification of the ovipositor using a dissecting light microscope (Leica Wild M3B Stereo). The bath was cooled or heated from 25 °C at a temperature change rate of 0.5 °C per minute till the critical thermal limits were ascertained. This rate of temperature change was selected to resemble that of field sites where *C. aquaticum* have been released. A freshly killed individual, with a 0.8 cm Type K thermocouple (Chromel/Alumel) inserted between the abdomen and the thorax, was used as a model to monitor rate of temperature change. The critical thermal limit temperatures were recorded when loss of coordination occurred and individuals which normally clung to the side of the vials, fell to the bottom of their vial and did not move. In addition, when removed from the water bath, loss of co-ordination was retested by placing the individual on its back immediately and stimulating the grasshopper with a fine brush to ensure the lack of activity was not voluntary. If a righting response or any movement was immediately noted then the individual was discarded from the data record. If lack of co-ordination was confirmed with the inability to right itself, then it was assumed it had truly reached its critical thermal limit. Recovery time was calculated as the time the individual was removed from the water bath to the point at which the individual could right itself when placed on its back. After recovering, the ten males and females were placed into a container (0.4 x 0.4 x 0.8 m) with fresh water hyacinth plants at 25 °C so that they could be retested. Individuals were retested after 72 hours to ascertain whether the previous test had depressed their critical thermal minima.

Acclimation trials

Critical thermal limits highlighted that winter temperatures would be more likely to affect the *C. aquaticum* population, as temperatures above the CT_{max} of

48 °C do not occur at water hyacinth field sites but winter temperatures do fall below the CT_{min} of 6 °C (Byrne et al., 2010). Therefore, only the lower temperatures were investigated further in acclimation and sub-lethal temperature trials. Twenty individuals (1:1 sex ratio) were removed from the main colony and placed into a container (0.4 x 0.4 x 0.8 m) with fresh water hyacinth plants. These containers were placed in a temperature-controlled room with a 12:12 L:D photoperiod and relative humidity at 50 ± 10 %. Adult grasshoppers were exposed to temperatures of 13 ± 2 °C, to simulate average early South African winter temperatures, for a period of 72 hours to acclimate them to colder conditions quickly. Thereafter, individuals were placed in a warm room (25 ± 1 °C) for one hour to adjust before critical thermal minima experiments were undertaken.

Sub-lethal survival trials

Sub-lethal temperature trials were carried out using individuals (n = 30) from the main colony that did not undergo the acclimation discussed above. Survival was tested after 24, 48 and 72 hours for the two temperature treatments and the 25 °C control. The two temperature treatments were 7 °C and 7.5 °C, representing an increase of 10 % and 15 % respectively of the critical thermal minima. Individuals were placed individually into separate empty vials (20 ml) sealed with moist cotton wool and placed into the water bath. The water bath was cooled to the temperature treatments and held for a duration of two hours. The individuals were then removed and placed into containers (0.4 x 0.4 x 0.8 m) at 25 °C with fresh water hyacinth to recover.

Data analyses

Statistical analyses were performed using Statistica (version 12.0; Statsoft, 2010) and IBM SPSS statistics 22 (IBM Corp., 2013). The data met the requirements for normality and therefore parametric tests were used. A Student's t-test was used to test for significant differences in critical thermal limits between sexes. A repeated-measures analysis of variance (ANOVA) was used to ascertain differences between the first and second measurements of the critical thermal minimum. A Student's t-test was used to test for significant differences in critical thermal minima and recovery time between the acclimated individuals and the stock individuals as well as the acclimated individuals and those that were retested. A linear regression was run to ascertain the extent to which critical thermal minimum and maximum are explained by body mass and sex. Differences in the survival between the control (25 ºC) and sub-lethal treatments (7 ºC and 7.5 ºC) were tested using a log-rank test, as the data was non-parametric. Differences in survival between males and females were tested using a Cox regression. If sex related differences were not noted, the individuals were pooled within treatments. The alpha value necessary to denote a significant difference was 0.05. Results are presented as mean ± standard error unless otherwise stated. The sample sizes used in the different experimental groups were largely based on the feasibility and accuracy of the acquired data, specifically the available resources at the time of experimentation and the size of the standard error.

Results

The critical thermal minima (CT_{min}) of females was not found to be significantly different than males in the stock (t = 1.16; df = 18; p = 0.26), retested (t = 1.11; df = 18; p = 0.28) and acclimated (t = 1.70; df = 18; p = 0.11; Table 2.1). No significant differences were found between male and female critical thermal maxima (t = 1.30; df = 18; p = 0.21).

Individuals acclimated to lower temperatures showed significantly lower critical thermal minima than stock individuals (Table 2.1; t = 3.54; df = 38; p = 0.001). Similarly, the critical thermal minima were significantly lower in acclimated individuals compared to those that were retested (t = 2.22; df = 38; p = 0.032). The results of the repeated-measures ANOVA indicated that retested individuals had significantly lower critical thermal minima 72 hours after the first CT_{min} tests (Wilks' Lambda = 0.709; F (1,19) = 7.790; p = 0.012; $n^2 = 0.291$).

Table 2.1. Critical thermal minima (CT_{min}) and maxima (CT_{max}) for *Cornops aquaticum* stock, acclimated and retested male (M) and female (F) adults.

Behavioural thermal parameters				
Population	Sex	n	CT_{min} (ºC)	CT_{max} (ºC)
Stock	M	10	6.29 ± 0.09^a	47.75 ± 0.47^d
	F	10	6.01 ± 0.08^a	48.24 ± 0.59^d
Retested	M	10	5.99 ± 0.08^b	-
	F	10	5.78 ± 0.10^b	-
Acclimated	M	10	5.35 ± 0.06^c	-
	F	10	5.74 ± 0.09^c	-

*Superscript letters represent statistically significant differences between means ($p < 0.05$).

The linear regression showed that body mass is a significant predictor of the critical thermal minima, but shows a weak relationship regardless of treatment (Figure 2.1; F = 9.624; p = 0.004). Survival in individuals at 7 °C was not found to be significantly different from the control after 72 hours (Figure 2.2; log rank test; $\chi^2 = 2.111$; df = 1).

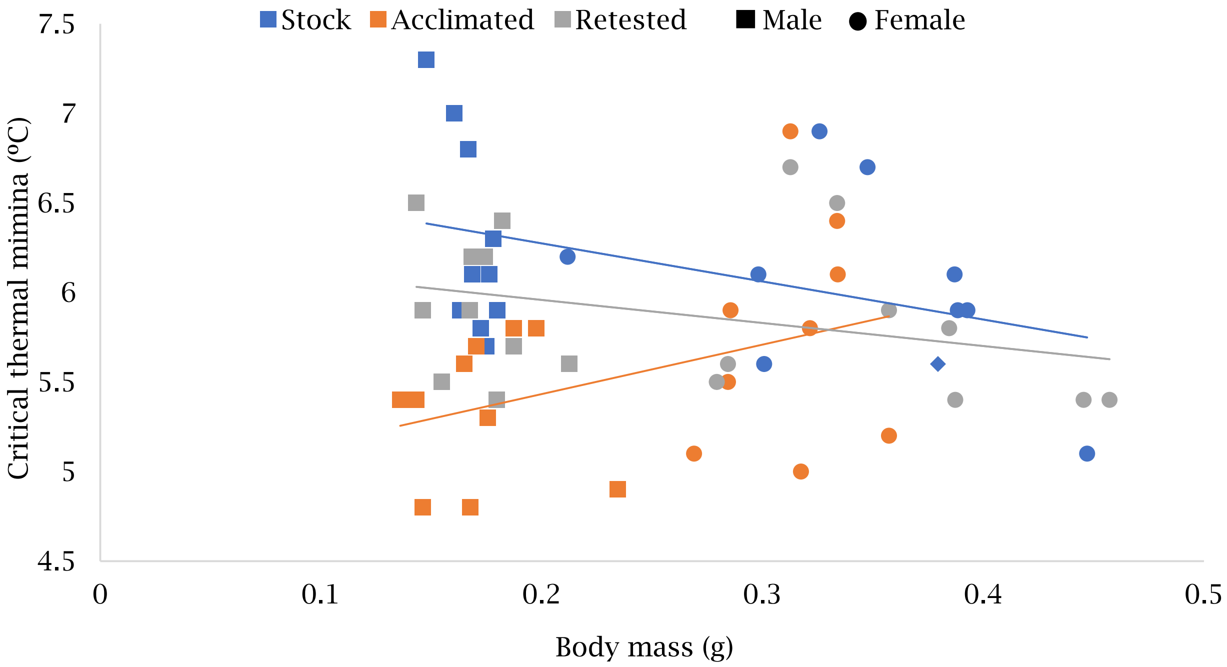


Figure 2.1. The relationship between body mass and critical thermal minimum between stock (blue; y = -2.13x + 6.70; R² = 0.16), retested (grey; y = -1.29x + 6.22; R² = 0.10) and acclimated (orange; y = 2.76x + 4.88; R² = 0.15) male (squares) and female (circles) *Cornops aquaticum* adults.

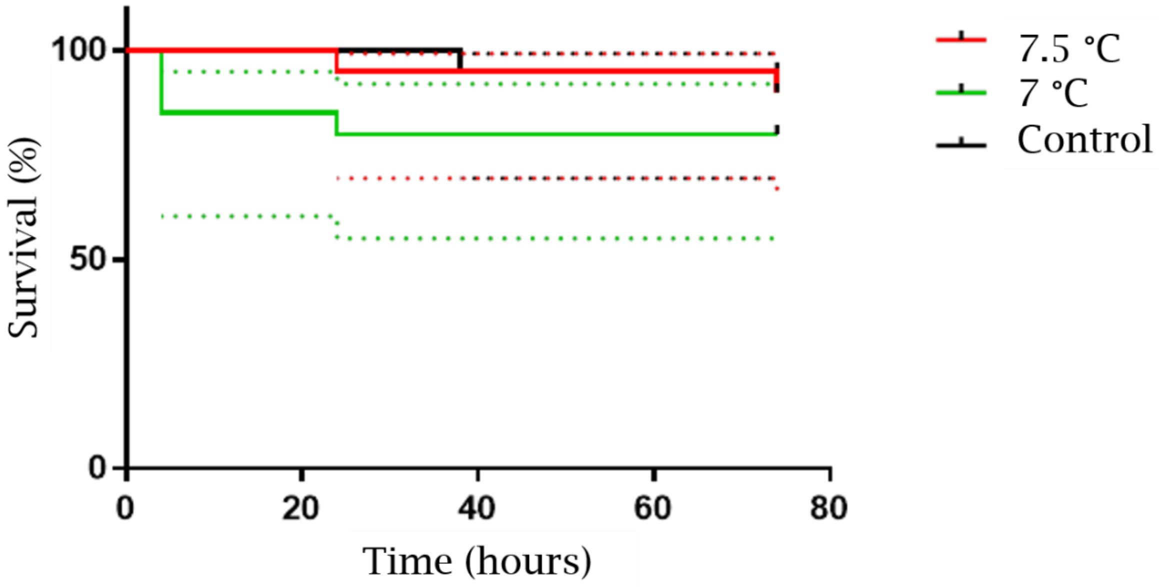


Figure 2.2. Survival curves of *Cornops aquaticum* adults subjected to three hours at 7 ºC, 7.5 ºC and 25 ºC as the control. Dotted lines signify 95 % confidence intervals.

Recovery time from chill coma was not found to be significantly different between males and females for the stock (t = 2.10, df = 18, p = 0.12), acclimated (t = 2.31, df = 18, p = 0.80) and retested individuals (t = 2.10, df = 18, p = 0.10). Recovery time after chill coma was found to be significantly quicker in the acclimated population compared to the stock individuals (Table 2.2; t = 2.414, df = 38, p = 0.02). Recovery time was not significantly different between acclimated and retested individuals (t = 1.52; df = 38; p = 0.136). Recovery time was not found to be significantly different between the stock and retested individuals (Wilks' Lambda = 0.912; F (1,19) = 1.8; p = 0.19; n^2 = 0.09).

Table 2.2. Mean recovery time ± standard deviation (seconds) after chill coma in stock, retested and acclimated *Cornops aquaticum* individuals. Superscripts denote significant differences between the means ($p < 0.05$).

Behaviour thermal measures	Treatments		
	Stock	Retested	Acclimated
Male recovery time (s)	141.5 ± 4.04^{a}	136 ± 10.83^{ab}	124.6 ± 10.31^{b}
Female recovery time (s)	169.7 ± 9.37^{a}	159.8 ± 6.50^{ab}	145 ± 5.98^{b}

Discussion

The CT_{min} is higher in *C. aquaticum* than in other tested biological control agents of water hyacinth. The water hyacinth mirid, *Eccritotarsus catarinensis* and water hyacinth mite, *Orthogalumna terebrantis,* have critical thermal minima of 1.2 °C ± 1.17 °C and 3.16 °C ± 0.52 respectively (Coetzee et al., 2007; Byrne et al., 2010; Marlin, 2011). Early measures of the water hyacinth weevil *Neochetina eichhorniae* has shown a CT_{min} that is closest to that of *C. aquaticum* at 4.3 °C however more recent estimates suggest the weevils have CT_{min} closer to *Eccritotarsus catarinensis* at 1.1 °C (Coetzee, unpub.; Reddy et al., 2019).

The critical thermal minima (CT_{min}) of approximately 6 °C suggests that at some sites of water hyacinth invasion, including Brede River and Crocodile River (*Cornops aquaticum* has not been released at either), *C. aquaticum* adults would go into chill coma and remain so for long periods of time, probably three to four hours during the night during winter (Byrne et al., 2010). These longer durations will likely cause considerable impairment to individuals and leave them vulnerable to predation or the possibility that an individual will fall into the water (Bale et al., 2002; Weisser and Siemann, 2013).

The critical thermal maximum (CT_{max}) of *C. aquaticum* of 47.75 ± 1.57 °C and 48.24 ± 1.96 °C, for males and females respectively, was similar to *N. eichhorniae* (51 °C) and *E. catarinensis* (49.6 °C ± 3.37 °C) and higher than *O. terebrantis* (44.52 °C ± 0.47 °C; Coetzee et al., 2007; Byrne et al., 2010; Marlin, 2011). This is likely due to the similarity of the habitat the biological control agents occur and the manner in which critical thermal maxima are tested (Rezende et al., 2011). It should be noted that these studies used a rate of temperature change of 1 °C/min. A faster rate of change did cause a higher CT_{min} in mosquitoes, *Anopheles gambiae* (Diptera: Culucidae) (Kelty and Lee, 1999; Terblanche et al., 2007). Therefore, the difference between the CT_{min} and CT_{max} values in these studies and the data presented could be even larger. The non-significant differences between males and females for the CT_{min} and CT_{max} suggest that body size and sex has a minimal effect on the thermal range in *C. aquaticum*. This is likely due to multiple factors determining the thermal range and is consistent with other studies (Hutchison, 1976; Lutterschmidt and Hutchison, 1997; Harris et al., 2015).

These preliminary results suggest sites such as Breede River, Kubusi River, Feesgronde, Warrenton Weir, Crocodile River and Delta Park would present problems for *C. aquaticum* as minimum daily temperatures during June and July are lower than the CT_{min} (Figure 2.3; Byrne et al., 2010). If *C. aquaticum* could acclimate to survive these temperatures, individuals would probably still have very low levels of activity during the day and thus, a similar risk of predation. Clairwood Quarry, Hammarsdale Dam, Mbozambo Swamp, Nseleni

River, Mkadhzi Spruit and New Years Dam are good candidate sites where *C. aquaticum* is unlikely to experience the risk of chill coma and increased predation during the winter months (Figure 2.3; Byrne et al., 2010).

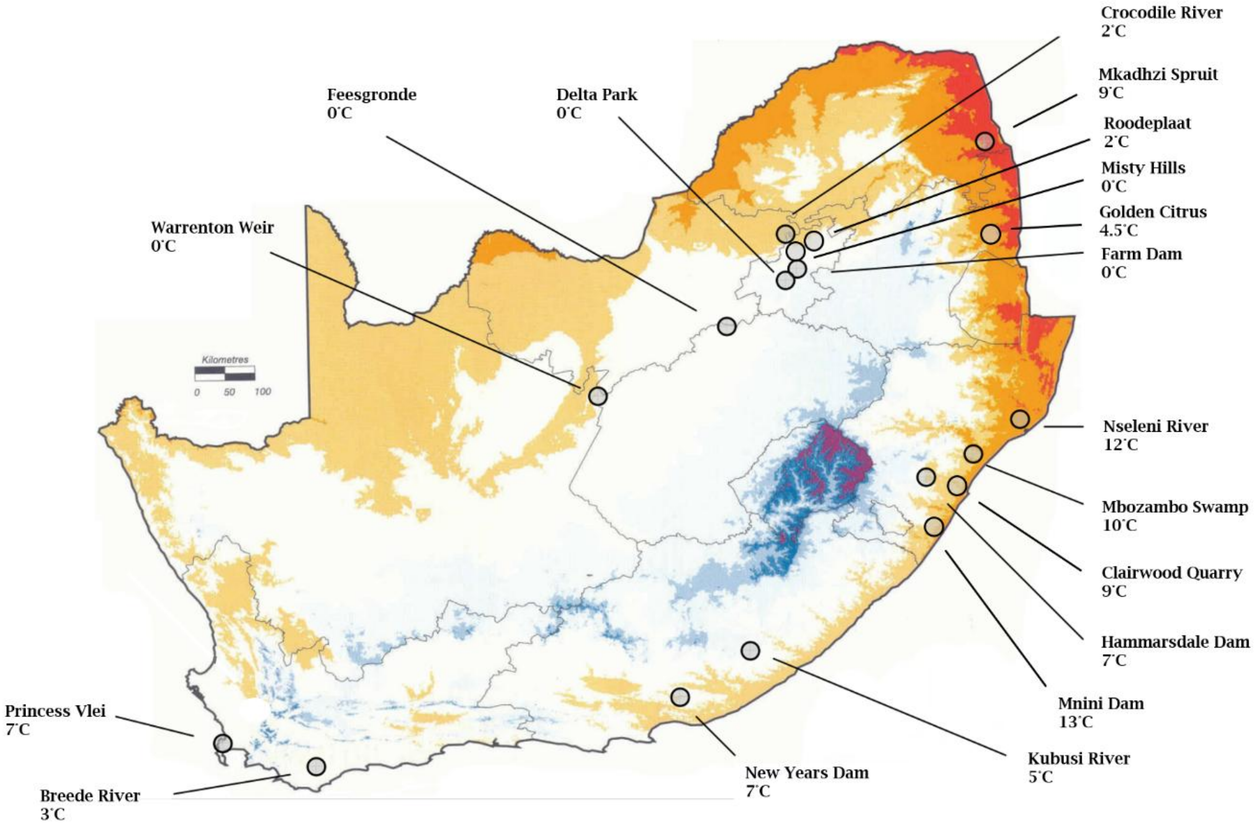


Figure 2.3. Map of mean annual temperature for South Africa ranging from < 8 °C (Purple) to > 22 °C (Red). Labelled on the map are 18 water hyacinth infested field sites with the lowest minimum daily temperature indicated below (adapted from Schulze and Maharaj, 1997; Byrne et al., 2010; data from ARC-PPRI).

In addition, the variation in the CT_{min} shown in the stock individuals and those acclimated is more restricted compared to other biological control agents. The variation of CT_{min} has been shown to be smaller than that of CT_{max} particularly at higher elevations (Chown, 2001). When compared to other biological control agents of water hyacinth, such as *Neochetina eichhorniae* (Coleoptera: Curculionidae), *C. aquaticum* had smaller variation in the measure of CT_{min} (Reddy et al., 2019). The variation noted in CT_{min} was also low when compared to other grasshopper species, such as *Kosciuscola cognatus*, *Kosciuscola tristis* and *Kosciuscola usitatus* (Orthoptera: Acrididae; Slatyer et al., 2016). The low amount of variation in the CT_{min} is probably a result of the reduced genetic diversity and variance of morphological characters present in the *C. aquaticum* population in South Africa compared to South America (Adis et al., 2008). This may either be a remnant of the original collection or as a result of laboratory breeding (Adis et al., 2008).

The comparison between variations in the CT_{min} between agents may not be objective, as although the methodological protocol's effect on the CT_{min} is at least partially understood, how it affects the variation of the critical thermal minima and maxima is not (Chown et al., 2007). However, the decrease of approximately 1 °C in the CT_{min} after a few days' acclimation is promising and notable but the degree to which *C. aquaticum* can extend its thermal range is yet unknown. In addition, the similar reductions in the CT_{min} after going into chill coma and recovering for three days shows the species can adjust within a relatively short space of time.

The fact that *C. aquaticum* recovers from chill coma faster after acclimation, but not after retesting suggests that individuals need a longer, more gradual thermal stress to improve recovery. The consistent but non-significant difference in the male recovery times compared to the female recovery times could be due to the smaller body size of the males. The smaller body size would allow males to return to ambient temperatures more quickly and thus allow a shorter recovery however, this should be investigated further. Even though body mass shows a weak causal effect on CT_{min}, the smaller individuals present in the South African population may have a reduced thermal range, or may not be able to acclimate to the same level, compared to those from the native distribution. The higher variation in body mass in female grasshoppers was likely due to grasshoppers at different stages of their egg development and their different sized fat stores (Weed-Pfeiffer, 1945; Uvarov, 1966; Berger et al., 2012).

In their native range, *C. aquaticum* uses the sedge *Oxycaryum cubense* (Poales: Cyperaceae) as a refuge when average temperatures drop during winter (Franceschini et al., 2011a; Figure 2.4). There are closely related sedges in South Africa, such as *Cyperus eragrostis* (Poales: Cyperaceae) and *Cyperus marginatus* (Poales: Cyperaceae) (Cilliers et al., 1998; Gordon-Gray et al., 2009). It is unknown if these plants are present at all water hyacinth sites and whether they provide a sufficient microhabitat to *C. aquaticum* (Gordon-Gray et al., 2009). This refuge could act as a buffer to allow acclimatisation when conditions change.

Figure 2.4. *Eichhornia azurea* (Liliidae: Pontederiaceae) floating meadow (foreground) and *Oxycaryum cubense* (background) floating mat at the Pampin Lake, Argentina (Franceschini et al., 2011a, pp 2).

In conclusion, acclimated individuals should go into chill coma at lower temperatures and recover faster, allowing them to avoid predators and not accumulate as much thermal stress. Whether or not this affects their survival is more complicated. The cost of acclimation to the individual could be a loss of performance over the thermal range (Gilchrist and Huey, 2001; Angilletta et al., 2002; Loeschcke and Hoffmann, 2002; Kristensen et al., 2008). The longer durations at sub-lethal temperatures suggest that individuals experiencing sub-lethal temperatures do not have increased mortality as a result of cold-induced injury. In addition, the degree to which *C. aquaticum* can acclimate, given repeated thermal stressors, is still unknown. The critical thermal minima of *C. aquaticum* provides the first step in understanding its thermal biology, and evaluating why establishment has been unsuccessful.

Chapter 3

Metabolic thermal range of *Cornops aquaticum* adults and immatures

Introduction

Biological control has the potential to provide a long-term solution to invasive plant problems. However, the control that biological control agents will provide is sometimes difficult to predict due to the fluctuating nature of the environment in which they are introduced (Coetzee et al., 2011). As a result, the success and efficacy of biological control relies on the exploitation of the complex interaction between the invasive alien plant (IAP) and its natural enemy. The long-term efficacy of the natural enemy is strongly affected by how, as a population, IAP species are impacted by the biological control agent and how population dynamics of both change spatially and temporally (Louda et al., 2003; Parshad et al., 2016). Species population levels and their fluctuations are chiefly controlled by how organisms interact with biotic and abiotic factors. This can be seen in water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae) whose growth rate is strongly influenced by water temperature and nutrient levels (Hauptfleisch, 2016).

An understanding of the physiology of an organism as it interacts with its environment should help identify important abiotic factors governing the species' abundance and distribution. The respiratory patterns displayed and metabolism are physiological features which provide quantitative data on the interaction of an organism with its environment (Addo-Bediako et al., 2002; Holtmann et al., 2017). Metabolism has been highlighted as a potential fundamental role-player in behavioural traits and life-history strategy (Kralj-Fišer and Schuett, 2014). The energy utilisation per unit time (referred to as metabolic rate) of an organism changes depending on the environmental variables acting on the organism and this can influence life-history characteristics such as egg development and feeding rates, determining the rate at which eggs are formed or the amount of biomass an insect can consume (Tewksbury and Deutsch, 2018). Although these factors are intertwined and difficult to separate, one potential way to quickly and effectively screen for the sensitivity of the organism is to test metabolic rates against known important factors, such as temperature (Chown et al., 2016; Williams et al., 2016).

Temperature is an important environmental variable known to strongly affect metabolic rates of exothermic organisms such as insects (Chown and Nicholson, 2004; Chown et al., 2016). The metabolic rate that *Cornops aquaticum* (Orthoptera: Acrididae) can maintain at different temperatures may explain how life-history characteristics such as development rates, feeding, longevity and fecundity may change with temperature particularly at different water hyacinth field sites (Slansky and Scriber, 1985; Roark and Bjorndal, 2009; Byrne et al., 2010). Other factors such as sex, body mass and age are also known to affect metabolic rates (Fielding and DeFoliart, 2008; Piiroinen et al., 2010). For example, metabolic rate was found to be significantly higher in male than female sand crickets (*Gryllus firmus* (Orthoptera: Gryllidae); Rantala and Roff, 2006). Understanding differences in thermal sensitivity of metabolic rate between the sexes can assist in the identification of potential problems with establishment, where the metabolic rates of females are severely diminished, especially at lower temperatures which limits egg production. The difference between the metabolic rate of male and female insects can be explained partially by the female's larger body size (Shillington, 2005). However, when controlling for body mass, male and female metabolic rates differ only when one sex has an energetically demanding activity or energy allocation that the other sex does not, such as egg development (Kolluru et al., 2004).

High metabolic rates seen in the female spider *Achaearanea tepidariorum* (Araneae, Theridiidae) are a prerequisite for producing larger clutches of eggs (Anderson, 1994). Rogowitz and Chappell (2000) showed that male eucalyptus-boring beetles (*Phorocantha semipunctata* and *Phorocantha recurva*) (Coleoptera: Cerambycidae) had higher metabolic rates regardless of mass and activity. Commonly an elevated metabolic rate in males is thought to be due to increased mate-seeking behaviours (Rogowitz and Chappell, 2000). Metabolic rate has been shown to increase through ontogeny and differ between different developmental stages, especially when undergoing sexual maturation as sexual organs become developed and utilise more energy (Elzen, 1986; Vogt and Appel, 1999; Snelling et al., 2011). For example, in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), the influence of temperature on the metabolic rate differs between workers and other castes (Vogt and Appel, 1999).

This response to temperature changes can also affect an organism after an initial thermal stress is experienced. For instance, a number of metabolic processes are altered in fluctuating thermal regimes, particularly after recovery from sub-zero temperatures (Lalouette et al., 2007). A proposed trade-off after a cold recovery is an increase in oxygen consumption which assists with cold damage repair but increases reactive oxidative species (Lalouette et al., 2007).

This can aid in understanding how metabolism changes when approaching thermal limits and the organism's optimal thermal range (Lalouette et al., 2011; Miller and Stillman, 2012). Understanding the effect of temperature on a key physiological parameter, such as metabolic rate, may provide valuable information on the biological control agent in question and thus assist in the selection and release strategy.

The manner in which metabolic rate changes in response to important environmental variables can highlight the biological control agents' developmental stages and sexes that are particularly sensitive and thus unlikely to respond well to different habitat conditions. A lower metabolic rate (%) at low temperatures may provide information on how, despite survival at low temperatures, performance could be hindered to the point of loss of condition. In addition, gas exchange patterns could also provide insight into *C. aquaticum* survival, as stressed individuals do appear to have more erratic respiratory patterns (Kestler, 1991).

Despite numerous releases, the water hyacinth grasshopper, *C. aquaticum*, has not established a population at any of the release sites in South Africa. This is largely thought to be a result of how *C. aquaticum* is interacting with the abiotic and biotic factors in South Africa. Temperatures at which metabolism is depressed need to be investigated in both adults and immature stages. The aim of this study was to record the metabolic rates for *C. aquaticum* adults and immatures at a range of temperatures which they will likely experience at water hyacinth field sites in South Africa.

Materials and methods

Study species

(Refer to Chapter 2 - Materials and methods, Study species and Chapter 1).

Experimental design and protocol

Respirometry experiments

Flow-through respirometry was used to measure individual grasshopper carbon dioxide (CO_2) emissions using a protocol adapted from Lighton (2008). Carbon dioxide concentrations (ppm) were logged using acquisition software (Expedata, Sable Systems v.1.1.14, USA). Air was pushed and pulled through the system using U-9900 and Elite E801 air pumps, with the flow rate monitored using a Hasting mass flow controller (CSI 500). Air was pulled at a constant flow rate of 60 $ml.min^{-1}$ through an infra-red CO_2/H_2O analyser (Licor LI-7000) to obtain per second intervals of CO_2 amounts. The system can be separated into an experimental system which passes over the specimen and the control system which acts as a baseline measurement (Figure 3.1). In the control system, air passed through one column of soda lime, one column of drierite and thereafter magnesium perchlorate to remove CO_2 and H_2O. In the experimental system, air was pushed through soda lime and a humidifier (continuous bubbling humidification; Vasu et al., 2008) to create CO_2 free humid air. The air should become fully saturated with water molecules at each respective temperature as it is bubbled through the humidifier (Zhang et al., 2011). This air was drawn into the respiratory chamber (100 ml^3) at a rate of 60 $ml.min^{-1}$ and out through a scrubber of magnesium perchlorate, to dry the air prior to entering the infra-red

CO_2/H_2O analyser (Licor LI-7000). *Cornops aquaticum* activity was monitored using an activity detector (Sable Systems AD-2-R).

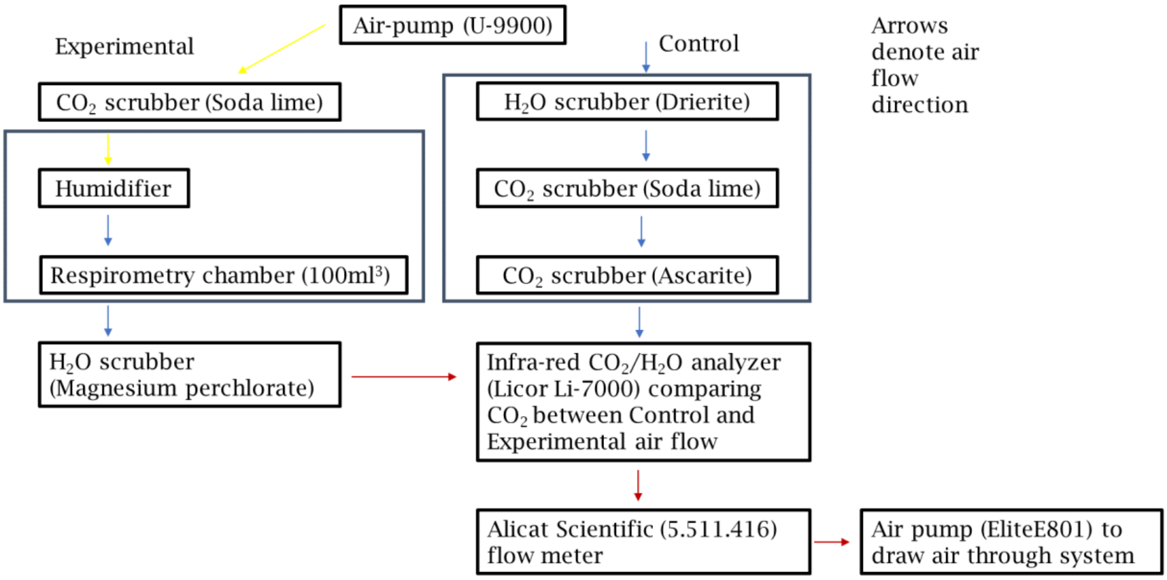


Figure 3.1. A representation of flow direction (arrows) and component set up of the flow-through respirometry system. The colours of the arrows denote the pump used (Green = U-9900; Blue = internal Licor Li-7000; Red = EliteE801).

Grasshoppers were housed individually in the respirometry chamber during the CO_2 measurements and placed in a Labcon low temperature incubator (Model L.T.I.E). Metabolic measurements were performed at 15 °C, 25 °C and 35 °C for both adults and immatures. Immatures were not sexed due to their lack of external characteristics and small size, instead *C. aquaticum* immatures were separated into three size classes based on mass to highlight early, intermediate and late immatures stages: < 0.05 g, 0.05-0.1 g and > 0.1 g. Individuals were weighed before and after the respiratory measurements using a semi-micro Shimadzu balance (Libror AEG-45SM; accuracy ± 0.00001 g). Carbon dioxide measurements were recorded for approximately two hours (7200 samples) with baselines preceding and following measurements. Five males and five females were used for the adult metabolic measurements at each temperature. Ten immatures for each temperature were tested with a minimum of three individuals per size class.

Data analyses

Drift corrections were performed on the baselines of the metabolic traces using Expedata (Sable Systems v.1.1.14, USA) to amend drift within the infra-red CO_2/H_2O analyser (Licor LI-7000). The metabolic trace was inspected to select portions at which CO_2 emissions were stable and the grasshopper was not moving (assessed using the activity detector) or stressed (assessed through frequent and erratic measurements) after which a minimum of 1000 CO_2 samples was used to calculate metabolic rate for that particular individual at the temperature being tested. *Cornops aquaticum* CO_2 production in parts per million (ppm) was converted to metabolic rate or VCO_2 (μl.hr^{-1}) in adults and mass-specific VCO_2 (μl.hr^{-1}.g^{-1}) in the immature grasshoppers using the following formulae:

$$VCO_2 = C(\frac{\text{FR (μl.}hr^{-1})}{1000}) \qquad \text{(Adults)}$$

$$VCO_2 = \frac{C(\frac{\text{FR (μl.}hr^{-1})}{1000})}{M} \qquad \text{(Immatures)}$$

Where FR is the flow rate of air through the system (ml. hr^{-1}), M is the mass of the individual and C is the CO_2 produced by the specimen in ppm (described in Lighton, 2008). The mass-specific metabolic rate was used in immatures as body mass was found to be a significant predictor. The average body mass prior to and after the respirometry experiments were utilised in the calculations of metabolic rate.

Statistical analyses were performed using Statistica (version 12.0) and IBM SPSS statistics 22. A linear regression was run to ascertain the extent to which adult metabolic rate was explained by body mass and sex. In addition, a linear regression was run to ascertain the relationship between body mass and metabolic rate for immatures at three different temperatures. Non-parametric tests were used as data would have required multiple data normalisation transformations. Kruskal-Wallis H tests were used to test for differences in the metabolic rate at different temperatures, for adults and immatures. A Kruskal-Wallis H test was used to investigate differences in average mass loss in adults during the respirometry experiment at different temperatures. No frass was noted in the respirometry chamber and thus mass loss was assumed to be equal to water loss. The alpha value necessary to denote a significant difference was 0.05. Results are presented as mean ± standard error unless otherwise stated.

Results

Metabolic measurements

No sex-specific differences were noted in the metabolic rates of adult *C. aquaticum* ($X^2_{(2)} = 20.49$; $p = 0.23$) but metabolic rate (VCO_2 µl.hr^{-1}) was significantly affected by temperature ($X^2_{(2)} = 20.49$; $p < 0.0001$; Figure 3.2).

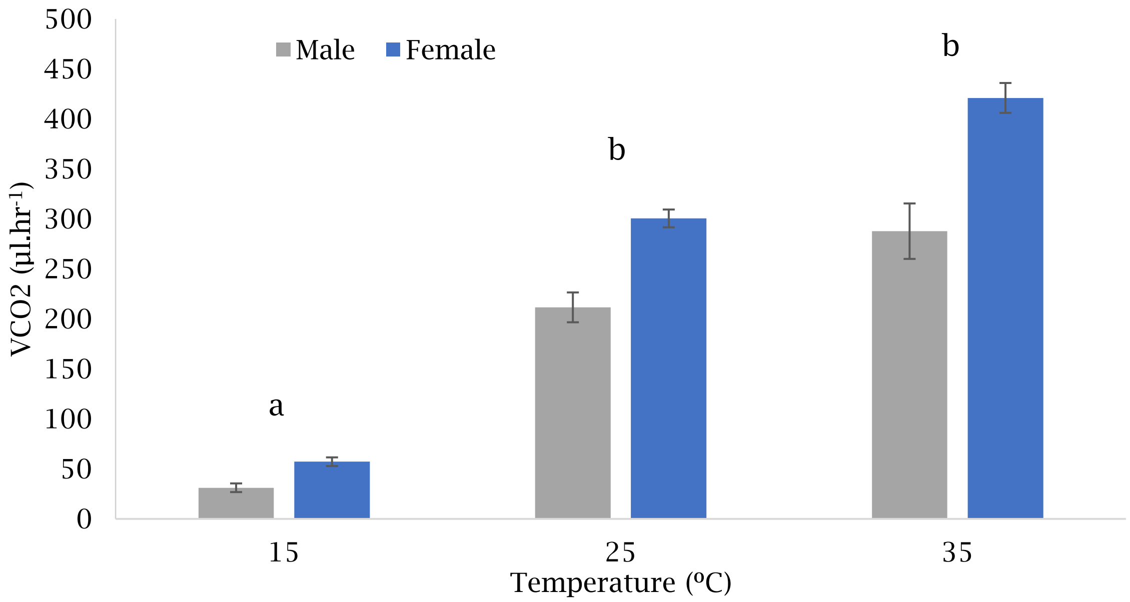


Figure 3.2. Mean ± SE (n = 5) metabolic rate or VCO2 (µl.hr-1) of Cornops aquaticum males and females at 15 ºC, 25 ºC and 35 ºC. Different letter above bars indicate a significant difference between means ($p < 0.05$).

As no significant difference in metabolic rate between the sexes was found, the results for males and females were combined. Although no significant difference was noted between metabolic rates at 25 ºC and 35 ºC, a Dunn-Bonferroni post-hoc test revealed both were significantly higher than the metabolic rates at 15 ºC ($X^2_{(2)} = 20.49$; $p = 0.003$ and $p < 0.001$ respectively). Metabolic rate showed a strong correlation with body mass at 25 ºC but not at 15 ºC or 35 ºC ($F_{(1;9)} = 7.26$; $p = 0.025$; Figure 3.3). *Cornops aquaticum* had a mass-specific metabolic rate of 1139.5 ± 89.97 µl.hr^{-1}.g^{-1} at 25 ºC. *Cornops aquaticum* males and females at 15 ºC exhibited only 15 % and 19 % of the metabolic rates they maintained at 25 ºC respectively. However, metabolic rates at 35 ºC were slightly higher than those displayed at 25 ºC but showed greater variation.

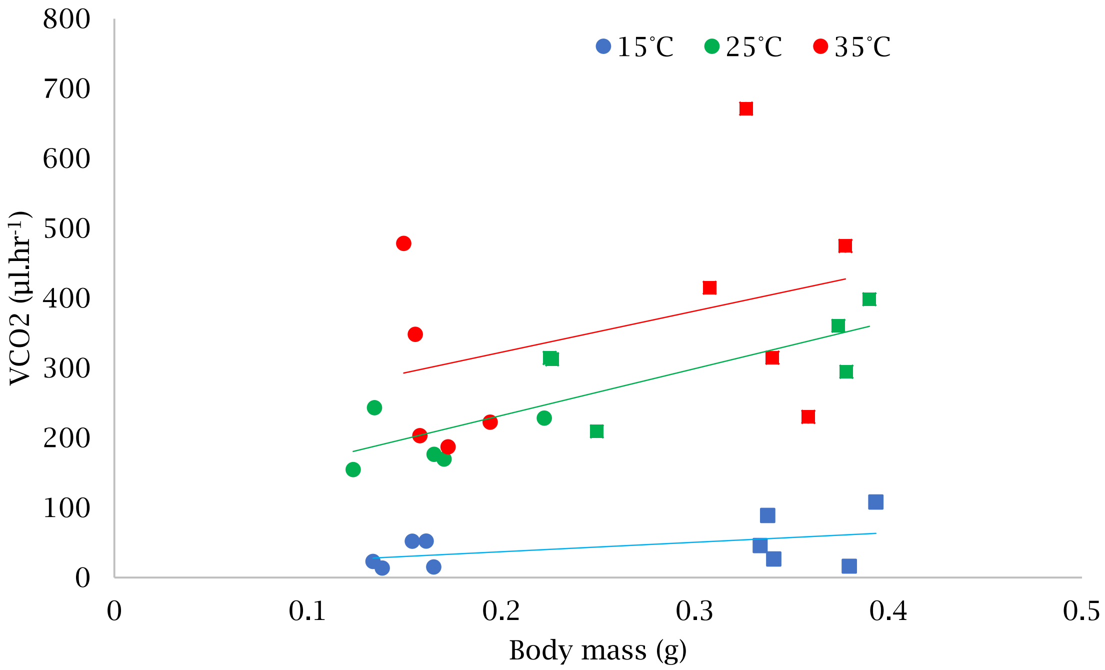


Figure 3.3. The relationship of body mass (g) and metabolic rate (VCO_2 µl.hr^{-1}) for adult *Cornops aquaticum* males (circles) and females (squares) at 15 ºC (y = 135.16x + 9.85 R² = 0.2117; p = 0.247), 25 ºC (y = 671.55x + 97.819; R² = 0,65; p = 0.025) and 35 ºC (y = 591.04x + 204.32; R² = 0.1304; p = 0.251) (n = 30).

Mass specific metabolic rate for immatures was significantly higher at 35 ºC than at 15 ºC and 25 ºC ($X^2_{(2)}$ = 19.613; $p < 0.001$ and p = 0.001 respectively; Figure 3.4). At 15 ºC and 25 ºC, immatures have metabolic rates 17 % and 19 % of that exhibited at 35 ºC. The immatures metabolic rates showed a strong relationship with body mass which strengthened with rising temperatures ($F_{15(1;8)}$ = 11.06; p = 0.01; $F_{25(1;8)}$ = 23.62; p = 0.001; $F_{35(1;8)}$ = 284.39; $p < 0.001$; Figure 3.5).

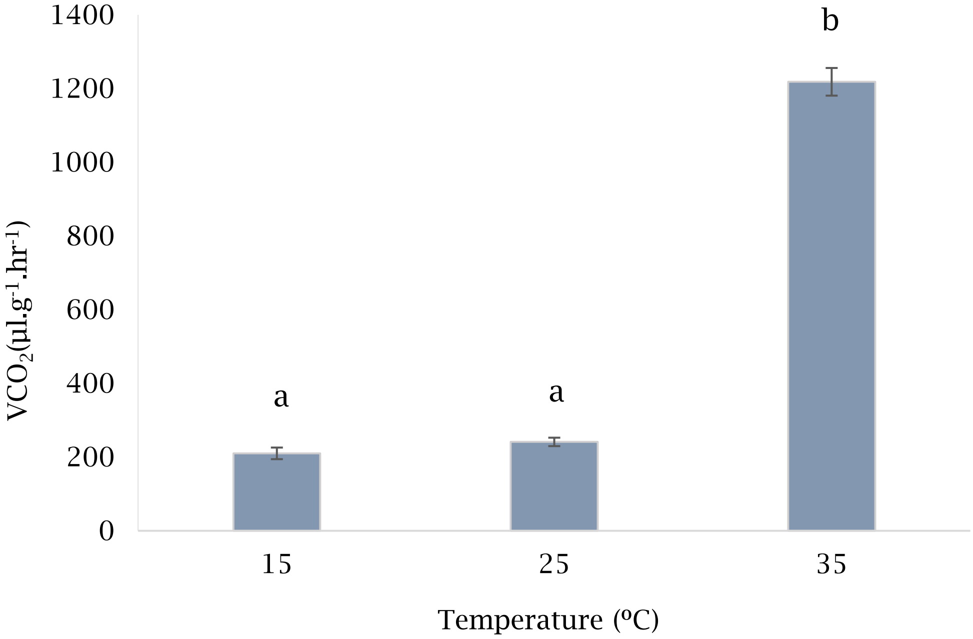


Figure 3.4. Mean ± SE (n = 10) metabolic rate or VCO$_2$ (µl.hr-^{1}.g^{-1}) of *Cornops aquaticum* immatures at 15 ºC, 25 ºC and 35 ºC. Different letters denote significantly different means (p < 0.05).

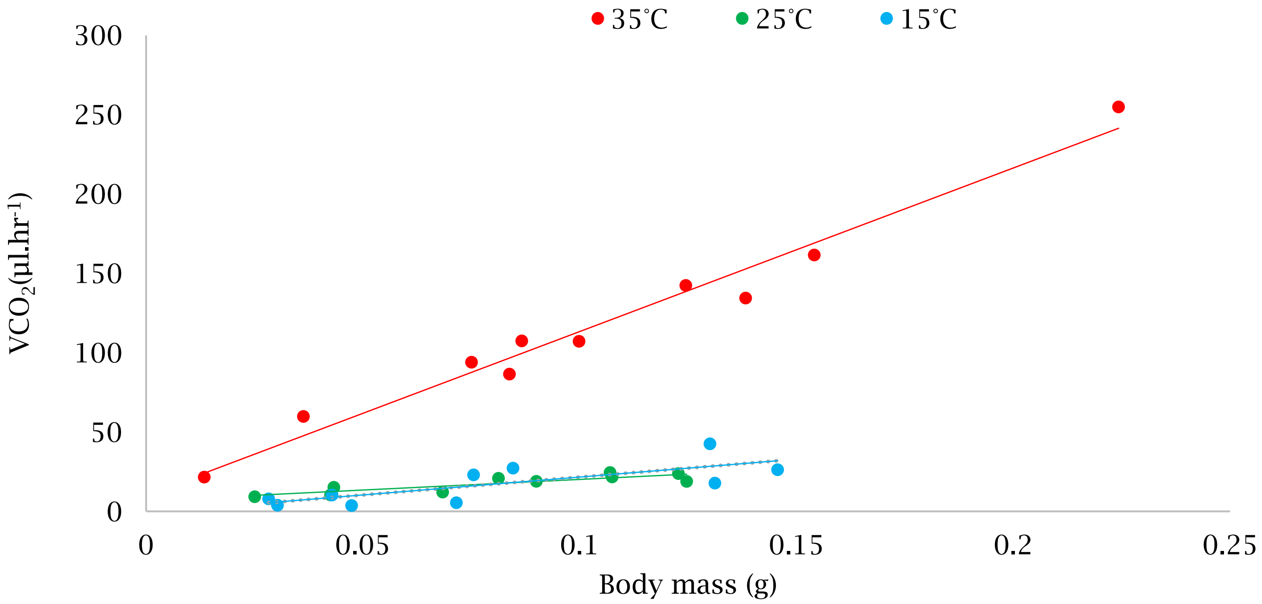


Figure 3.5. The relationship between body mass (g) and metabolic rate (VCO$_2$ µl.hr^{-1}) for *Cornops aquaticum* immatures at 15 ºC (y = 224.85x - 0.75; R² = 0.58; p = 0.01), 25 ºC (y = 134.8x + 6.76; R² = 0.75; p = 0.001) and 35 ºC (1030.4x + 10.32; R² = 0.97; p < 0.0001).

Mass loss

Cornops aquaticum body mass was 0.1599 ± 0.0066 g (n = 15) and 0.3335 ± 0.014 g (n = 16) for males and females, respectively. During the respiratory measurements average mass loss per hour was 6.402e-4 ± 2.03e-4, 2.245e-3 ± 6.60e-4 and 3.918e-3 ± 6.15e-4 at 15 ºC, 25 ºC and 35 ºC, respectively (Figure 3.6). This corresponds to a potential percentage water loss of 0.47, 1.87 and 3.60 for 15 ºC, 25 ºC and 35 ºC, respectively. Average mass loss per hour during the respiratory measurements was significantly different between the different temperatures ($X^2_{(2)} = 19.404$; $p < 0.0001$; Figure 3.6). Mean mass loss per hour at 15 ºC was significantly lower than that at 25 ºC ($p = 0.041$) and 35 ºC ($p < 0.0001$), as indicated by a Dunn-Bonferroni post-hoc test. Effect size analyses indicate that temperature accounted for 67 % of the variability of mass loss per hour.

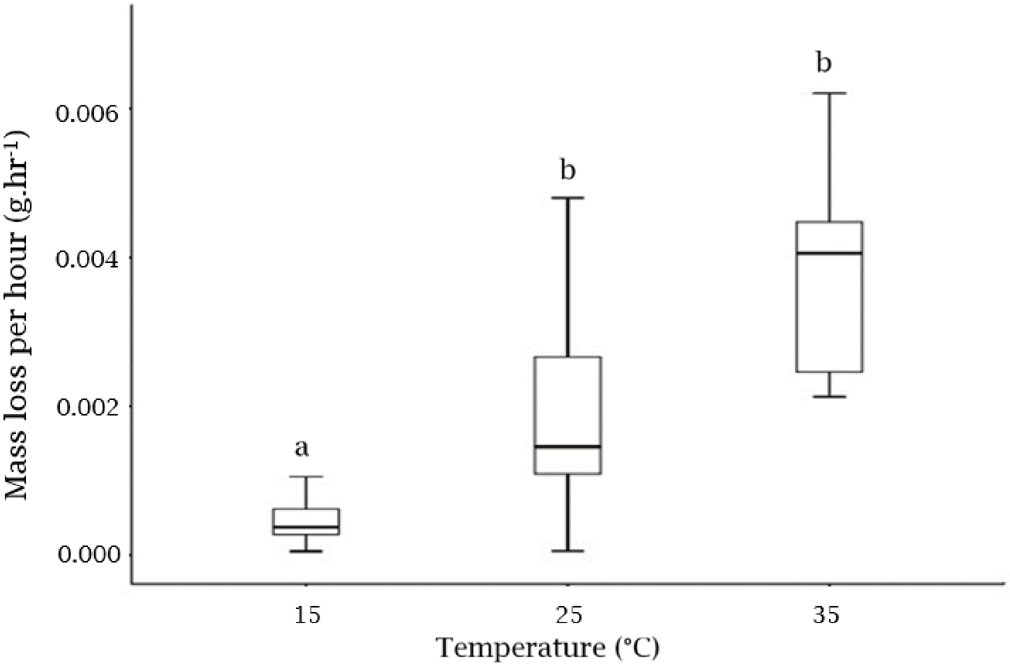


Figure 3.6. Mass loss per hour of *Cornops aquaticum* at 15 ºC, 25 ºC and 35 ºC indicating the medians, range and interquartile range (n = 31).

Gas exchange

Continuous and cyclic gas exchange patterns were generally seen in *C. aquaticum* adults at 15 ºC, 25 ºC and 35 ºC (Figure 3.7). Cyclic ventilation patterns with evidence of abdominal pumping were also observed with discontinuous gas exchange noted in only one female at 35 ºC (Figure 3.8). *Cornops aquaticum* immatures showed similar cyclic ventilation patterns with abdominal pumping at the different temperatures, however, no discontinuous gas exchange patterns were noted.

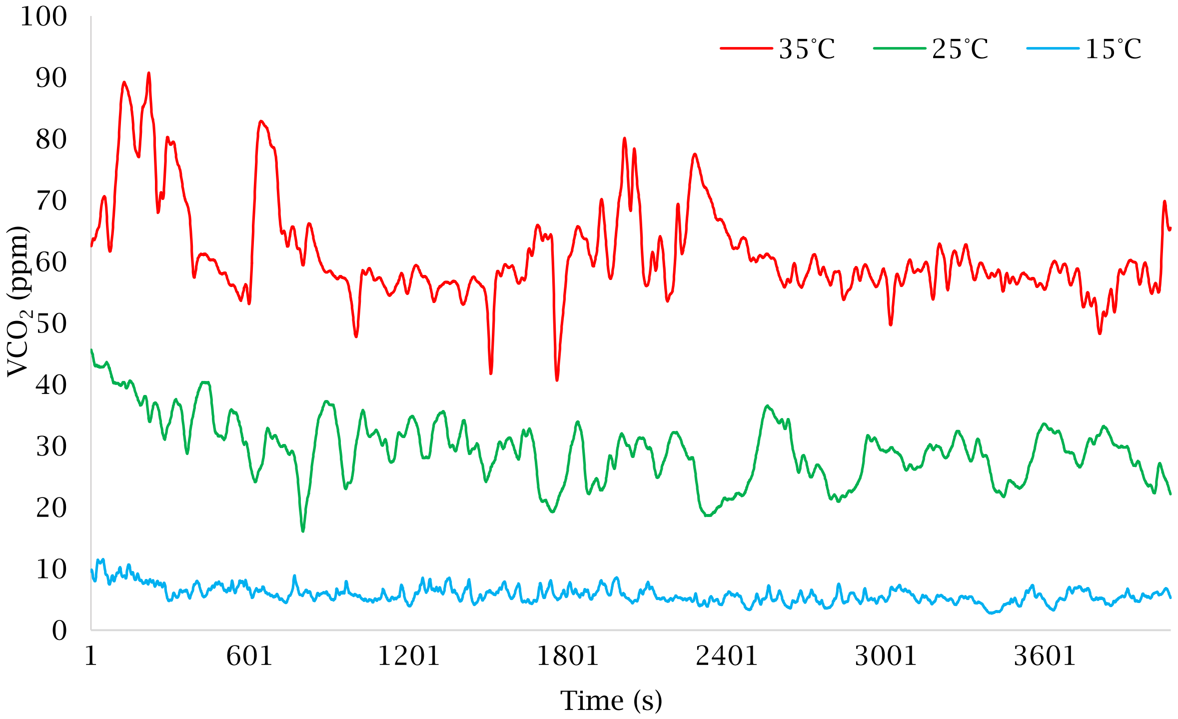


Figure 3.7. Portion of three different *Cornops aquaticum* adults' carbon dioxide production measurements in parts per million at 15 ºC, 25 ºC and 35 ºC.

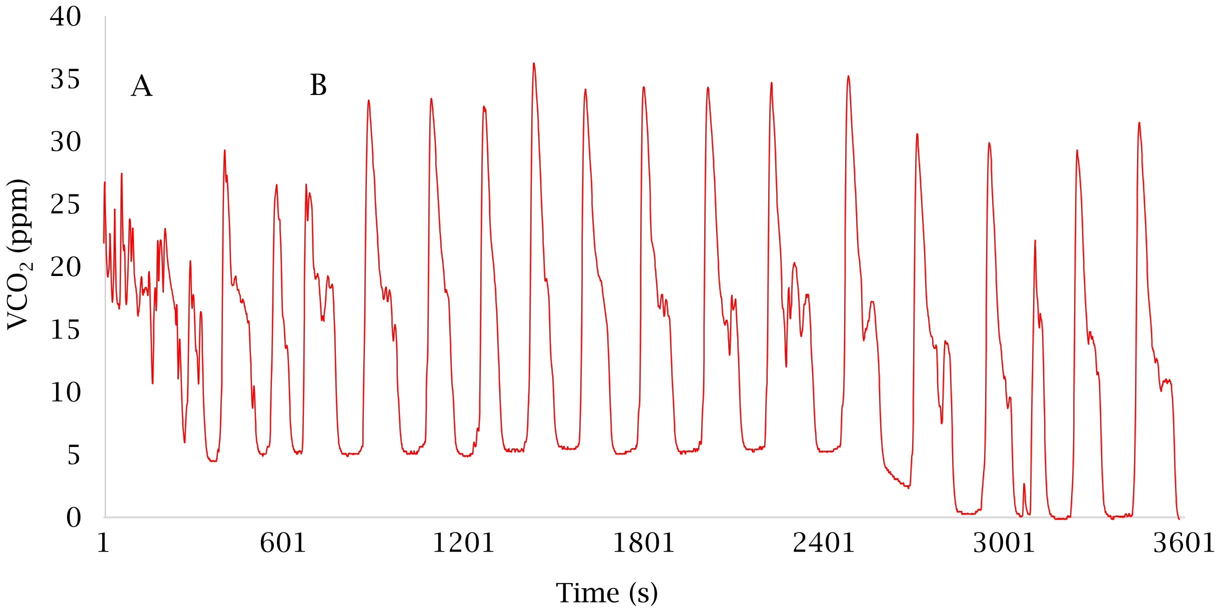


Figure 3.8. *Cornops aquaticum* carbon dioxide production measurements in parts per million shown by only one adult female at 35 ºC showing discontinuous gas exchange patterns. The letters A and B denote activity and abdominal pumping respectively.

Discussion

Compared to other related species, *C. aquaticum* has by far the highest mass-specific metabolic rate for their body size at 25 ºC (Table 3.1). *Cornops aquaticum* appears to have a metabolism which is optimised for competition and predation avoidance but may be costly to conserve energy during winter conditions (Menge and Sutherland, 1987; Clarke, 1993; Reinhold, 1999).

The high metabolic rate at 25 ºC does seem appropriate given the average daily temperatures at points along the Amazon River such as Manaus at 27.92 ºC and Santarem at 26.72 ºC (Climex and Dymex Suite 4.0). Therefore, high metabolic rates and the likely increased activity associated with them would be beneficial in their native range. South African sites like Misty Hills and Crocodile

River where mean daily canopy temperatures (ARC monitoring protocol) can drop to 8.27 °C and 11.74 °C, respectively, from May to June, would likely result in a severe long-term depression of the metabolic rate of adults. This could be problematic as it increases the risk of lower energy stores and a high mortality risk (Roark and Bjorndal, 2009; Krams et al., 2013). Overall reactions of *C. aquaticum* to a reduced metabolic rate may range from attempts to thermoregulate to maximising energy savings (Guderley and St-Pierre, 2002; Storey and Storey, 2012).

Table 3.1. Mean resting mass-specific metabolic rates (µl.hr^{-1}.g^{-1}) ± SD (when available) of closest-related species to *Cornops aquaticum* at the tested temperatures.

Species	Common name	Resting metabolic rate (testing temperature)	Reference
Melanoplus sanguinipes	California grasshopper	214 ± 34 (25 °C)	Rourke, 2000
Hophlosphyrum griseus	Cricket	473 ± 190 (27 °C)	Nespolo et al., 2003
Schistocerca gregaria	Desert locust	414 (27 ± 2 °C)	Armstrong and Mordue, 1985
Schistocerca americana	American locust	896 (35 °C)	Greenlee and Harrison, 2004
Locustana pardalina (gregarious)	Brown locust	Males 227 ± 6 (25 °C) Females 239 ± 13 (25 °C)	Duncan and Hanrahan, 2018
Locusta migratoria	Migratory locust	649 ± 44.8 (35 °C)	Snelling et al., 2011
Xanthippus corallipes	Acridid grasshopper	1292 ± 395 (35 °C)	Ashby, 1997
Romalea guttata	Eastern lubber grasshopper	156 (25 °C) 240 (29 °C)	Hadley and Quinlan, 1993 Quinlan and Hadley, 1993
Taeniopoda equez	Horse lubber grasshopper	310 (29 °C)	Quinlan and Hadley, 1993
Arphia conspersa	Speckled rangeland grasshopper	400 (22 °C) 823 (32 °C)	Forlow and Macmahon, 1988
Arphia pseudonietana	Red-winged grasshopper	443 (22 °C) 774 (32 °C)	Forlow and Macmahon, 1988
Trimerotropis pallidipennis	Pallid-winged grasshopper	395 (22 °C) 749 (32 °C)	Forlow and Macmahon, 1988
Cornops aquaticum	Water hyacinth grasshopper	1139.5 ± 89.97 (25 °C)	This study

The similarity in metabolic rates at 25 ºC and 35 ºC suggests the preferred temperature of adults likely falls between these temperatures. However, the higher metabolic rate at 25 ºC may be an adaptation to maximise metabolic rate, and thus other LHCs at the temperatures lower than their preferred range (Williams et al., 2016; Ploomi et al., 2018). The similarity may be an adaptation to the laboratory conditions in which they were reared (Auer et al., 2015). The reduced metabolic rate at 15 ºC suggests that even during mild conditions, grasshoppers will likely have largely reduced activity compared to those maintained at 25 ºC. The low metabolic rate at 15 ºC highlights that *C. aquaticum* metabolism is sensitive to colder temperature, however whether this would change if the duration *C aquaticum* was exposed to the temperature was increased is not known (Zhu et al., 2016). A depressed metabolic rate is a key aspect to adaptation to colder environments, however the fact that such a reduced metabolic rate is occurring at a mild temperature suggests the *C. aquaticum* is suited to warmer conditions and may accumulate high thermal stress over the course of the South African winter (Guppy, 2004; Laloutte et al., 2011; Kelly et al., 2014).

Based on the low metabolic rate and low activity of *C. aquaticum* adults at 15 ºC, the Misty Hills site may not be warm enough for the grasshopper to persist during the winter months as it is close to their critical thermal minima and adults will likely lose condition through winter conditions (Lalouette et al., 2011; Chapter 2). In addition, the temperatures would likely affect feeding rates and the ability to avoid predators (Krams et al, 2013). However, the manner in which metabolic rates increase between 15 ºC and 25 ºC may allow persistence during the colder months at the Golden Citrus site. For instance, if *C. aquaticum* metabolic rates at 20 °C are 80 % that of those noted at 25 °C then winter temperatures of 20 °C may be enough to allow individuals to feed and develop normally. During summer, when temperatures are generally above 25 °C, metabolic rates suggest there should be no loss of *C. aquaticum* performance in South African water bodies, however, a direct comparison of metabolic rates should be made between native South American and South African populations, given the low genetic diversity of the South African population (Adis et al., 2008). The non-significant difference in the metabolic rates between males and females at 15 °C, 25 °C and 35 °C suggests both sexes have high energy expenditure behaviours, likely mate seeking behaviour and egg production (Weed-Pfeiffer, 1945; Quinlan and Hadley, 1993; Rogowitz and Chappell, 2000; Kolluru et al., 2004).

The metabolic rate of the immature *C. aquaticum* suggests much lower growth rates at temperatures equal to and below 25 °C, with higher temperatures being necessary for fast development. It is likely that immatures require a more stable warm spring and summer to flourish. Future studies should compare this metabolic rate range to the preferred temperatures of *C. aquaticum* immatures and adults and the early field temperatures in spring at South African water hyacinth sites, compared to the native range. A large discrepancy would lead to reduced activity, lower growth rates at colder temperatures and likely increase basking behaviour and with this, predation risk (Onsager, 2000; Samietz et al., 2005; Whitman, 2008). The much higher metabolic rate at 35 °C may indicate the grasshopper's reliance on higher temperatures for development.

Mass loss variation was strongly influenced by temperature and was quite substantial given that humid air was used in the respiratory chamber. However, overall percentage water loss during testing was low compared to the eastern lubber grasshopper, *Romalea guttata* (Orthoptera: Romaleidae), at 2.1 %, 3 % and 3.8 % for 15 °C, 25 °C and 30 °C, particularly at lower temperatures (Quinlan and Hadley, 1993). The relative water loss, given that humid air was used, supports *C. aquaticum*'s semi-aquatic lifestyle and association with water hyacinth. This will strongly affect how individuals would survive separated from their host plant even for as short a time as two hours. Further studies should monitor the movement of *C. aquaticum* individuals on a water body. Gas exchange patterns did not seem to change between the different temperatures as compared to other studies (Bradley, 2007; Contreras and Bradley, 2010) with the only observed discontinuous gas exchange cycle seen at 35 °C.

Chapter 4

Cornops aquaticum preferred body temperature and outdoor population monitoring

Introduction

Insect distribution, abundance and evolution are influenced by climatic variables among other factors. Of these variables, temperature has a particularly strong effect (Régnière et al., 2012; Yang et al., 2015). Temperature can affect biochemical processes in many ways, such as altered enzyme activity and the rate of chemical reactions (Fand et al., 2015). These reactions control life-history characteristics (LHCs) such as development, fecundity and survival (Musolin, 2007; O'Connor et al., 2011). Thus, a deeper understanding of how these LHCs influence populations can assist biological control attempts through improved species selection, rearing and establishment. Life-history characteristics are often linked strongly to a species' fitness and thus their evolution (Gotthard et al., 1994; Buckley et al., 2017).

Fitness can be loosely defined as the ability of a species to propagate itself through survival and reproduction (Hansen, 2018). Therefore, fitness of a species is generally influenced by a range of characteristics such as development time, overall female size and number of eggs produced (Mäenpää and Smiseth, 2017). These characteristics also give insight into the geographical distribution of species and its potential as a biological control agent. This can be noted in parasitoid wasps *Peristenus digoneutis* and *Peristenus stygicus* (Hymenoptera: Braconidae) where their high fecundity resulted in them being reconsidered as biological control agents (Haye et al., 2005).

The potential change in species' LHCs resulting from climate change further complicates biological control efforts (Parmesan et al., 2000; Kingsolver et al., 2013; Cohen et al., 2018). The frequency of pest outbreaks is predicted to increase as climate fluctuations become more common thus making current biological control systems more unpredictable (Stireman et al., 2005; Reeves, 2017). Species are predicted to follow their preferred climatic conditions where possible or will be required to adapt to local conditions (Gerard et al., 2013; Bonamour et al., 2019).

Thus, populations must deal with increased environmental variation either through local adaptation or with phenotypic plasticity (Whitman and Agrawal, 2009; Stoks et al., 2016). Phenotypic plasticity is defined here as a particular genotype exhibiting different phenotypes depending on the conditions they experience (Arnold et al., 2019; Pigliucci, 2001). Phenotypic changes, either arising from phenotypic plasticity or genetic selection, are likely important when a species experiences new environmental conditions, as is common in biological control programmes (Merilä and Hendry, 2014; Qin et al., 2017; Ziv et al., 2017). For example, in the red-legged grasshopper, *Melanoplus femurrubrum* (Orthoptera: Acrididae), populations exhibited phenotypic plasticity through an increased thermal sensitivity when moved from Connecticut to Vermont (Rosenblatt et al., 2016). Other species, of which *Drosophila* (Diptera: Drosophilidae) has been studied to the greatest degree, have shown that rising temperatures can impact numerous traits such as broader thermal limits, faster chill coma recovery, higher reproduction and lower mortality (Gerken et al., 2016; Hoffmann et al., 2003; Kristensen et al., 2008; Xue et al., 2019). However, aforementioned changes rely on the genetics of the species. Phenotypic plasticity is a trait that evolves through natural selection based on genetic variation (Via and Lande, 1985; Acasuso-Rivero et al., 2019).

Biological control systems are complicated by the fact that despite being a low cost and chemical free alternative for pest management they work with more unknowns than mechanical and chemical control (Roderick et al., 2012). The biological control of invasive alien plants (IAPs) deals with two major difficulties. The first is the invasive species has naturalised and its distribution range differs from its native one (Roderick et al., 2012). The potential microevolution or range expansion of the invasive species and the reason for dispersal can make control efforts ineffective. The second is the action of collecting individuals from the invasive species' native range and the subsequent transportation and rearing of said individuals can limit the genetic diversity of the resultant population. The rearing of these populations is generally conducted in quarantine facilities where they remain while host specificity testing and release approval is conducted (Bownes et al., 2010b, 2011). The effect on the genetic variation likely depends on the survival during transportation and the number of years the biological control agent remains in a quarantine or rearing facility.

Low genetic diversity, through genetic bottlenecks, can reduce the ability of a population to tolerate new environmental conditions and thus makes the establishment of a new population or maintenance of population numbers difficult (Fauvergue et al., 2012). A genetic bottleneck is here defined as a significant loss of genetic diversity normally from a large reduction in the population size over a single generation (Szűcs et al., 2017). The size of a bottleneck and the number of bottlenecks a population undergoes has been correlated with lowered fitness (Briskie and Mackintosh, 2004; Jamieson et al., 2006). Thus, small founding populations and populations where numbers are more stochastic in nature can create multiple bottlenecks and increase local extinction (Frankham, 1997; Hartl and Clark, 2007; Leberg and Firmin, 2008). For instance, the metapopulation of desert locust (*Schistocerca gregaria*) is thought to persist in their solitarious phase between swarms/plagues, experiencing local extinction events and bottlenecks (Chapuis et al., 2014). However, their overall genetic diversity remains high due to the sheer number of individuals in the overall metapopulation (Chapuis et al., 2014). As such, founding populations with greater genetic variation have been shown to be more successful (Kephart, 2004; Vergeer et al., 2005). Smaller population sizes can also be positive as it removes negative alleles from the population, for example the ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) required multiple releases for establishment in North America but once established it invaded globally (Lombaert et al., 2010).

These genetic bottlenecks, limited genetic variation or laboratory selection can at least be somewhat mitigated by “renaturalising” the population (Taylor et al., 2011). The term “renaturalising” is defined here as the reintroduction of natural environmental factors to an individual or population. A crucial component to mitigate the negative components of poor genetic diversity seems to be a rapid expansion of the population to restore genetic variation (Nei et al., 1975; Frankham, 2005). For example, the water hyacinth mirid bug, *Eccritotarsus catarinensis* (Hemiptera: Miridae), was kept under laboratory conditions for only four years, but the population underwent a severe bottleneck while in quarantine resulting in the release population to be founded by a single gravid female (Martin Hill, pers. comm.; Taylor et al., 2011). To mitigate these effects, a population of 1000 individuals were collected from their native range and were later rereleased. Laboratory populations of rereleased *E. catarinensis* were successfully "naturalised", exhibiting improved critical thermal limits (Taylor et al., 2011; Porter et al., 2019). The critical thermal minima of the "renaturalised" wild population was -0.3 ± 0.063 ºC compared to laboratory specimens 1.1 ± 0.054 ºC (Porter et al., 2019). This provides a base for other biological control species that may exhibit lower genetic diversity compared to their native range. Lower genetic variation as a result of genetic bottlenecking can therefore, at least partially, be replenished (Taylor et al., 2011).

Cornops aquaticum (Orthoptera: Acrididae) is a semi-aquatic neotropical grasshopper, released in South Africa in January 2011 (Bownes and King, 2010; Bownes et al., 2011) to control the invasive aquatic plant water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae). In its native range *C. aquaticum* population dynamics are influenced by the quantity of its host plants and their seasonal change (Franceschini et al., 2007), however this has not been confirmed to influence the South African population. *Cornops aquaticum* populations in quarantine in South Africa have been shown to have lower genetic diversity, have reduced wing size and be morphologically smaller than most South American populations (Brede et al., 2008). This may have occurred as a result of the original collection not being a true representation of the South

American population or laboratory rearing bottlenecking the genetic diversity, or a combination of the two (Baker et al., 2003; Franks et al., 2011). The genetic diversity of *C. aquaticum* is likely be substantially reduced after almost 25 years in quarantine. The general loss of natural resilience of populations with low genetic diversity (Frankham, 2005), the time under laboratory conditions and the lack of any true establishment at any site suggests *C. aquaticum* has had substantial losses in fitness compared to native specimens. The addition of genetic diversity, either through recollection of native populations or "renaturalisation" of the species, is probably necessary to improve the likelihood of establishment through the increased potential to adapt to new conditions (Lee, 2002; Roman and Darling, 2007; Dlugosch and Parker, 2008).

If climatic constraints are the key as to why *C. aquaticum* has not established, then the monitoring of the species with specific respect to their body temperature in response to ambient temperature is vital. Monitoring outdoor populations of insects involves a fair amount of estimation and with that, uncertainty in the results (Milner-Gulland and Shea, 2017; Schultz et al., 2017). The manner in which populations are affected by winter temperatures, particularly at higher latitudes, and the actual daily ambient temperature fluctuations experienced by individuals can negatively affect development and fecundity (Bale et al., 2002; Vasseur et al., 2014). This offers an opportunity to better understand how the thermal biology of *C. aquaticum* may hinder its establishment.

Temperature variability has been shown to potentially impact fitness through the non-linear nature of thermal reactions as well as to amplify effects of increased temperature on insect survival (Deutsch et al., 2008; Paaijmans et al., 2013; Greenlee et al., 2019). For example, *Drosophila melanogaster* (Diptera: Drosophilidae) experience a positive population growth when temperatures fluctuate around an average of 17 °C, but a negative growth rate when the same variation occurs at an average of 24 °C (Bozinovic et al., 2011). Thermal performance curves partially assist in this as they display graphically how temperature affects performance (Clusella-Trullas et al., 2011).

These thermal performance curves can highlight the potential optimal temperature for performance and potentially development, fecundity and disease resistance (Angilletta, 2006; Bakken and Angilletta, 2014). An organisms' ideal performance is thought to occur at their most frequently encountered temperature (Preston and Johnson, 2020). However, this temperature may be different for different life-history characteristics. As a result, certain life-history characteristic may be optimized at the cost of reduced performance of others. For example, the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae), has been shown to select temperatures that enhance growth rates, at approximately 38 °C, at the cost of reduced nutritional efficiency (Miller et al., 2009). Disease can be rampant in grasshopper populations and create limits on population density, however disease in grasshoppers normally occurs in early developmental stages and does not cause host death (Hudson et al., 2002; Elliot et al., 2005; Kistner and Belovsky, 2014). The resistance to disease may rely on a complex interaction of environmental temperatures, thermal fluctuations and both host and pathogen thermal sensitivity (Thomas and Blanford, 2003; Springate and Thomas, 2005). For example, *Locusta migratoria* select higher temperatures (>40 °C), to prevent mycosis while infected with the entomopathogenic fungus *Metarhizium* *anisopliae var. acridum* (Hypocreales: Clavicipitaceae) (Ouedraogo et al., 2004; Sangbaramou et al., 2018). This extends the lifespan of the locust at the cost of increased metabolism, lowered growth rates and decreased fecundity (Gardner and Thomas, 2002; Elliot et al., 2005). As such, the preferred temperatures an organism selects must be viewed in conjunction with the potential trade-offs.

Measurements of body temperature preference in insects have been performed since 1922 (Deal, 1941). The recent use of non-invasive methods, such as thermal cameras, may assist in reducing the artificialness of previous methods (Deal, 1941; Clissold et al., 2013). The thermal imaging does require an appropriate calibration for emissivity, atmospheric pressure, air temperature and humidity. Emissivity is the degree at which radiation is given off an object compared to that of a blackbody, with plants generally ranging from 0.92 and 0.99 (Jones, 2004; López et al., 2012; Rakrueangdet et al., 2016). Temperature measurements based on thermal radiation allow the specimen as well as part of the environment to be sampled (Childs et al., 2000). In addition, thermal cameras may allow certain natural behavioural responses at various temperatures to be observed (Harris et al., 2015). For example, in the lubber grasshopper, *Taeniopoda eques* (Orthoptera: Romaleidae), preferred body temperature was maintained under varying temperatures and solar radiation through body posture adjustments (stilting, vertical orientation, flanking, crouching and stem-shading) and microhabitat selection (Whitman, 1987). This may be of particular importance as water hyacinth plants likely have multiple microhabitats which depending on the position of *C. aquaticum* in relation to its host plant may be important to buffer local environmental conditions, such as cold temperatures.

The selection of microhabitats and the timing of these selections can highlight how preferred temperatures are maintained above ambient conditions and the potential costs incurred from these behaviours (Samietz et al., 2005; Harris et al., 2015). Microhabitat selection such as the selection of a particular substrate (the surface on which the specimen rests) can be key as different substrates can possess varied thermal properties but likely also different predator risk based on camouflage (Karlsson et al., 2009). In the pygmy grasshopper, *Tetrix undulata* (Orthoptera: Tetrigidae), different colour morphs showed varying degrees of habitat selectivity based on maintenance of preferred body temperature and predator avoidance (Ahnesjö and Forsman, 2006).

Cornops aquaticum has been released at ten different sites in South Africa. Grasshoppers were observed for a few months at some of the release locations, but with decreasing numbers as time progressed (Anthony King, pers. comm.). Closer monitoring of *C. aquaticum* population dynamics over time, especially through winter may give a better understanding of survival and potential resurgence of the population. In addition, movement of the released individuals at establishment sites and predation are not typically considered in the release of the biological control agents (Heimpel and Asplen, 2011; Pratt and Center, 2012). Thus, implementation of monitoring with the focus on the effect of temperature requires the population to be enclosed in a certain area, to prevent movement and limit predation.

Therefore, the main aim of this chapter is to monitor a caged outdoor population of *C. aquaticum* over time, in addition to investigating the body temperature of *C. aquaticum* in relation to its’ substrate temperature and its’ position relative to its host plant at different ambient temperatures. The objectives will be to, 1) at a population level, try to "renaturalise" an outdoor population of *C. aquaticum* while monitoring population numbers and the local canopy temperatures, 2) investigate the actual temperatures of the grasshoppers (adult and immatures) and their host plant (substrate) and 3) track the location of *C. aquaticum* in relation to their host plant.

Materials and methods

Experimental design and protocol

Outdoor Cornops aquaticum population

Fifty *Cornops aquaticum* individuals were removed from inside the temperature-controlled rooms of the University of the Witwatersrand Insectary and Quarantine Facility (Chapter 2) in May 2017 and placed in a 93 cm x 111 cm x 45 cm container covered with a fine mesh cloth. The container was filled with 45 large water hyacinth plants, resulting in an insect to plant ratio of 1.11:1. This container was housed in a larger 200 cm x 300 cm x 200 cm mesh cloth container outdoors in the University of Witwatersrand Nursery, to exclude predators such as birds. The temperature conditions for this location, based on average temperature and lowest minimum temperature, would be most similar to the Golden Citrus site (Figure 2.3). Water hyacinth plants were replaced every two weeks from water hyacinth ponds at the University of the Witwatersrand during spring and summer, and from those in the insectary during the winter. Predators, mainly spiders, were actively removed when plants were replaced. An additional 80 individuals, from the South African Sugarcane Research Institute

(SASRI), were added to the population after the count on the 9th of December 2017 was performed. Thus, the outdoor population consisted of a mixture of individuals from SASRI and University of the Witwatersrand. Water and canopy microhabitat temperatures were monitored using Thermochron® iButton® devices (DS1921G Maxim Dallas Semiconductor Corporation). Daily average ambient temperature was obtained using weather data for Parktown (South African Weather Service) and the aforementioned iButton® devices. Population counts were performed in two stages. Firstly, a disturbance of the water hyacinth was performed to move a portion of the insects to the fine mesh netting ceiling of the cage. A second horizontal mesh was then placed over the water hyacinth separating individuals that had moved in response to the disturbance and those that had not. An intensive search was conducted on the water hyacinth in order to find individuals hiding between water hyacinth plants. The mesh was then slowly removed counting individuals that had been initially separated. The total population number, without differentiating between *C. aquaticum* adults and immatures, were counted every 2-4 weeks over a period of 15 months. Any predators that were observed during the counting of the population were removed.

Cornops aquaticum thermal images

Cornops aquaticum adults and immatures were taken from inside the temperature-controlled rooms of the University of the Witwatersrand Insectary and Quarantine Facility culture (Chapter 2) and placed on outdoor water hyacinth ponds at the University of Witwatersrand Greenhouse. The grasshopper and substrate temperatures were recorded using the FLIR E60 infrared camera (FLIR systems Inc. Wilsonville, Oregon, USA), while a Type K thermocouple (Chromel/Alumel) measured the ambient temperature. In addition, a log of the behaviour responses was noted to investigate patterns linked with temperature and the life stages. Grasshopper position was also recorded and characterised into 5 categories: “Above leaves”, “Below leaves”, “Petiole”, “Base of the plant” and “Off of plant”. Thermal images for body temperature of adults and immatures as well as their respective substrates were recorded during spring and early summer (September to December) and winter (July and August) to encompass conditions similar to those experienced by immatures emerging earlier or later in the season. Thermal images were recorded approximately every two hours, from 7h00 to 17h00 and 8h00 to 16h00 for immatures and adults respectively. An emissivity value (ε) of 0.987 was used to estimate the ratio of thermal radiation by the plant material compared to a blackbody in order for accurate temperature readings to be acquired.

Data analyses

The instances of grasshopper position were tested for normality with a Shapiro-Wilk test. A chi-squared goodness test for independence was performed to test if grasshopper adults and immatures, varied their position based on their body temperature. A Pearson correlation and linear regression were computed to assess the relationship between body temperature and the spring and winter substrate temperatures in both adults and immatures. The alpha value necessary to denote a significant difference between means was 0.05. Results are presented as mean ± standard error unless otherwise stated.

Results

Outdoor Cornops aquaticum population

An outdoor population of *C. aquaticum* was monitored for a period of 15 months from 25 April 2017 to 25 July 2018, encompassing one summer season and two winter seasons. The outdoor population persisted over the two winters, although the number of *C. aquaticum* individuals dropped considerably during May and July in both 2017 and 2018, constituting reductions of approximately 60 % and 68 % in population size (Figure 4.1). Despite the addition of 80 individuals in December 2017, the population numbers dropped to levels similar to those noted in June 2017. The mortality (23 %) between February 2018 and April 2018 were observed to largely consist of the *C. aquaticum* immatures which had hatched during December 2017 and February 2018 (G. Venturi, pers. obs.). Daily temperature (mean ± SD) during spring and winter was 20.1 ± 2.1 °C and 11.2 ± 2.4 °C respectively. The daily average canopy temperature for the first cold fronts experienced by the outdoor population were 11.6 ± 3.2 °C (4 June - 7 June 2017) and 9.33 ± 0.6 °C (14 May to 16 May 2018). The average evening temperatures (18h00 to 6h00) during these cold fronts were 7.1 ± 1.0 °C and 5.8 ± 1.3 °C respectively. Browning of water hyacinth plants was noted at sites that were fed on by *C. aquaticum* which contributed to the general browning of water hyacinth and quickly resulted in reduced plant quality.

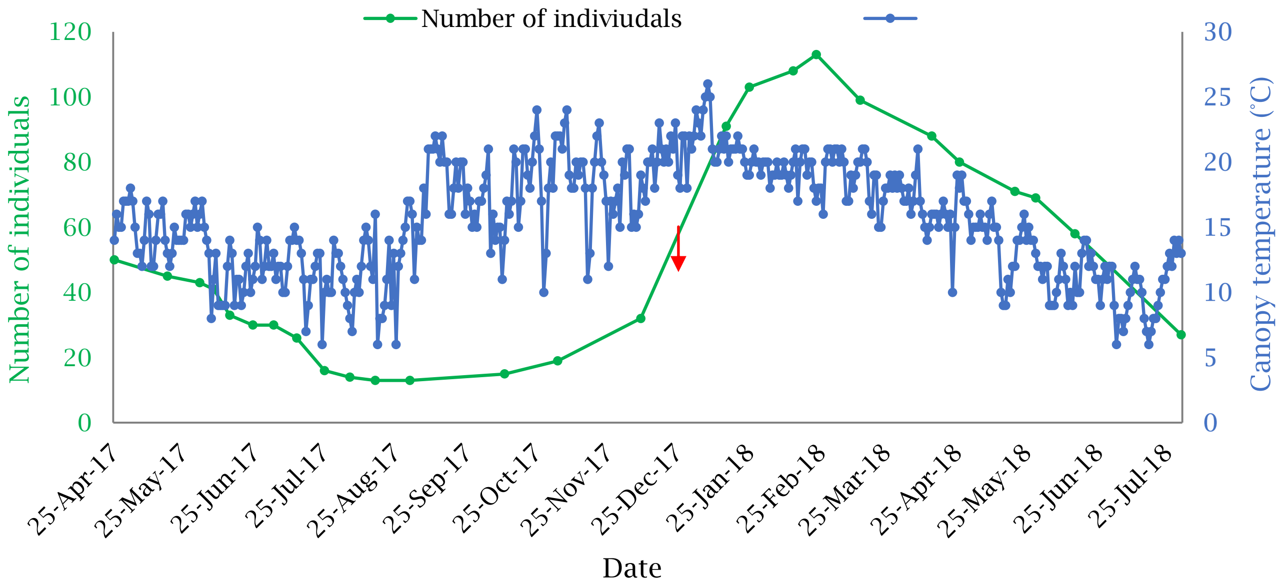


Figure 4.1. Number of *Cornops aquaticum* adults and immatures and the daily canopy temperatures detected over time in an outdoor cage. The red arrow (↓) represents when 80 individuals were added to the outdoor cage.

Cornops aquaticum thermal images

Adult and immature *C. aquaticum* exhibited different substrate selection at varying temperatures. There was a significant association between adult grasshopper body temperature and their position relative to water hyacinth ($X^2_{(12)}$ = 88.03; $p < 0.001$) (Figure 4.2). The position of adult grasshoppers relative to water hyacinth varied considerably, but at colder ambient temperatures (11 - 15 °C) adults were more likely to be found at the base of the plant (33.1 %) or off their target plant (39.3 %). At ambient temperatures above 19 °C adults were more likely to be found at the petiole (31.6 %) and on the upper leaves (31.1 %) of the plant.

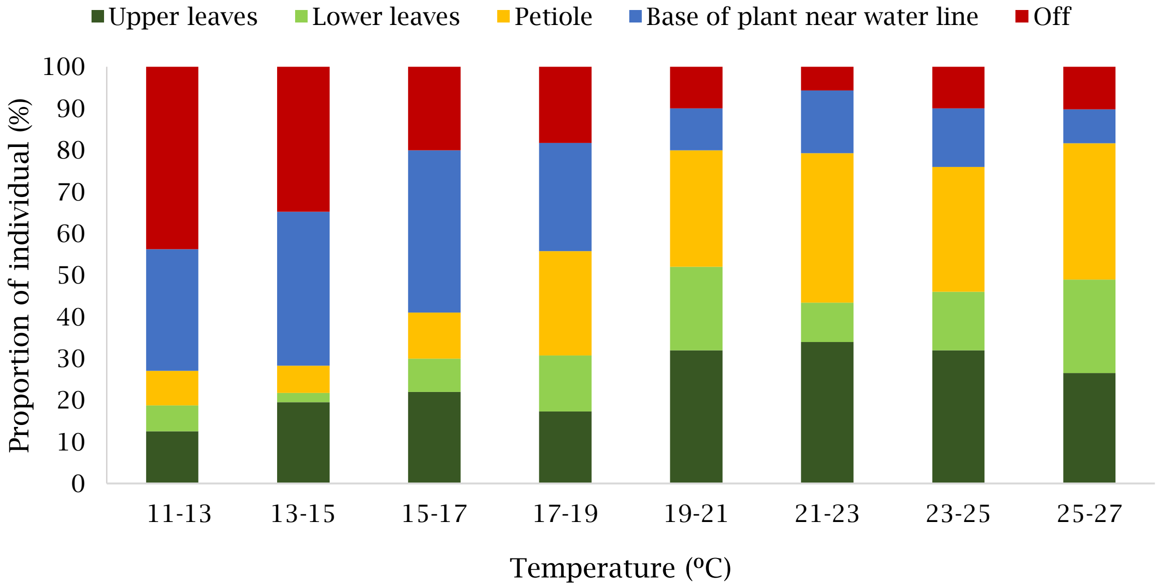


Figure 4.2. Proportion (%) of the *Cornops aquaticum* adult population found on different parts of the outdoor water hyacinth ponds. Positions were recorded between 8h00 and 16h00 at temperatures ranging from 11 ºC to 27 ºC with n > 46 for each temperature range.

Immatures were found to also have an association with temperature and position relative to water hyacinth ($X^2_{(12)} = 58.14$; $p < 0.001$; Figure 4.3). *Cornops aquaticum* immatures at colder ambient temperatures (11 - 15 ºC) were more likely to be found at the base of the plant (26.4 %) or on top of the leaves (29.1 %). At ambient temperatures warmer than 17 ºC, immatures spent the majority of their time on the upper leaves (70.8 %).

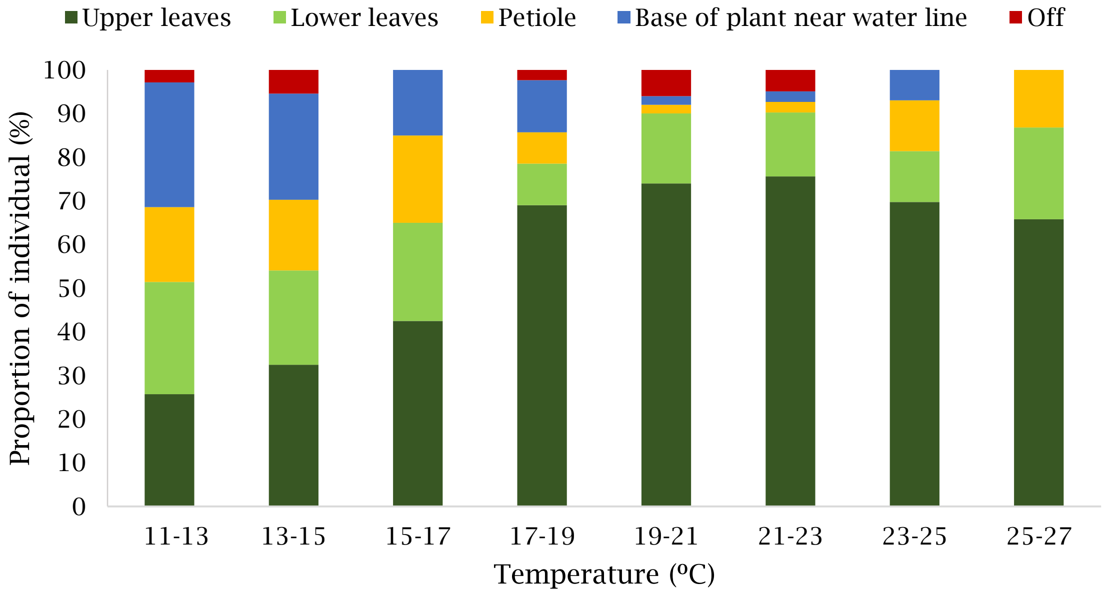


Figure 4.3. Proportion (%) of *Cornops aquaticum* immatures population found on different parts of the outdoor water hyacinth ponds. Positions was recorded between 7h00 and 17h00 at temperatures ranging from 11 ºC to 27ºC with n > 36 immatures for each temperature range.

Two behavioural responses were noted in immatures of *C. aquaticum* when predators approached, immatures would at first remain relatively motionless when approached on the top of leaves but would and jump when the predators came too close (G. Venturi, pers. obs.). Although jumping was noted in response to movement by the observer and predators, behaviour of the immatures was generally more sedentary, particularly at colder periods of the day. Adults made active attempts to hide under leaves or behind petioles and actively jumped when disturbed (G. Venturi, pers. obs.). Feeding was noted infrequently, occurring between 15 ºC and 39 ºC in adults and 19 ºC and 30 ºC in the immatures.

The body temperatures of *Cornops aquaticum* adults were generally found to be warmer than the water hyacinth substrate on which they perched (Figure 4.4a, b, c; Figure 4.5). Whilst *C. aquaticum* immatures body temperature was similar to water hyacinth substrate (Figure 4.4 d; Figure 4.6). Although no significant correlation between substrate temperature and adult *C. aquaticum* body temperature was noted during spring ($F_{(1;29)} = 2.26$; $p = 0.143$; Figure 4.5), substrate temperature had a significant effect on *C. aquaticum* adult body temperature during winter ($F_{(1;29)} = 56.1$; $p < 0.001$; Figure 4.5).

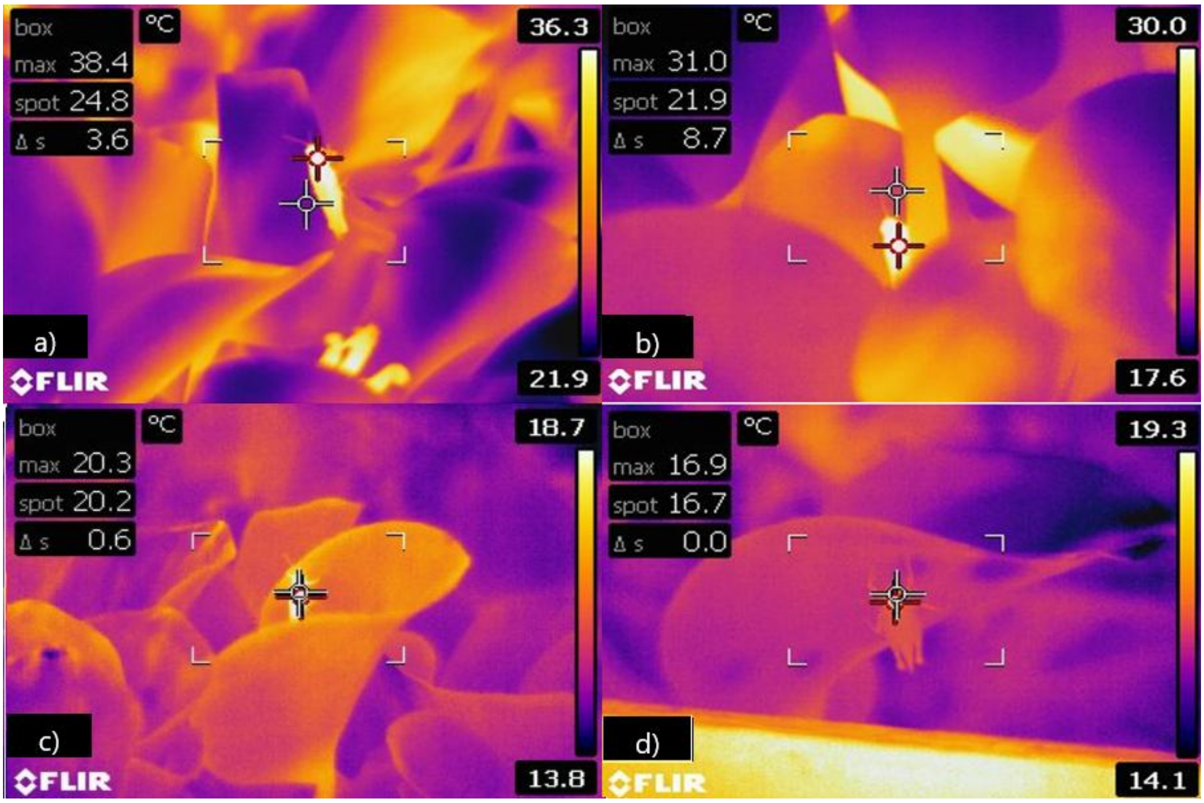


Figure 4.4. Thermal images of *Cornops aquaticum* immatures and adults on their host plant, water hyacinth during spring (a and b) and winter (c and d). a) Adult located on upper leaves. b) Adult located on the petiole. c) Adult on the upper leaves. d) Immature located on the lower leaves. The red crosshairs (max) indicates the hottest points within the white rectangular focal areas (box), the white crosshairs (spot) indicate the centres of the images and the delta (Δ s) signs indicate the differences between the max and spot measurements.

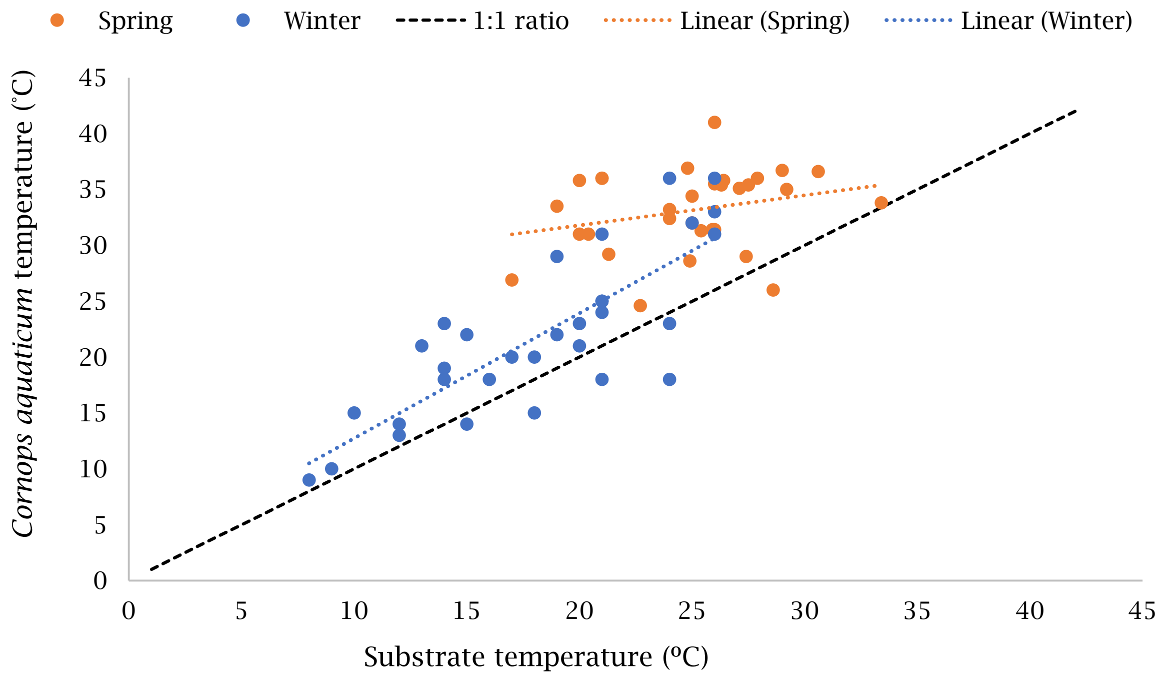


Figure 4.5. Relationship between substrate temperature (ºC) and Cornops aquaticum (ºC) adult body temperature during spring (orange) ($y = 0.2716x + 15.932$) ($R^2 = 0.073$; $p = 0.143$) ($n = 31$) and winter (blue) ($y = 1.1194x + 1.5413$) ($R^2 = 0.6592$; $p < 0.001$) ($n = 31$). The black dashed line depicts a 1:1 ratio between body temperature (ºC) and substrate temperature (ºC).

Immatures of *C. aquaticum* generally maintained body temperatures closer to substrate temperature particularly at earlier times of the day. Substrate temperatures had a significant effect on immatures' body temperature at both spring ($F_{(1;24)} = 76.59$; $p < 0.001$) and winter temperatures ($F_{(1;24)} = 133.03$; $p < 0.001$; Figure 4.6).

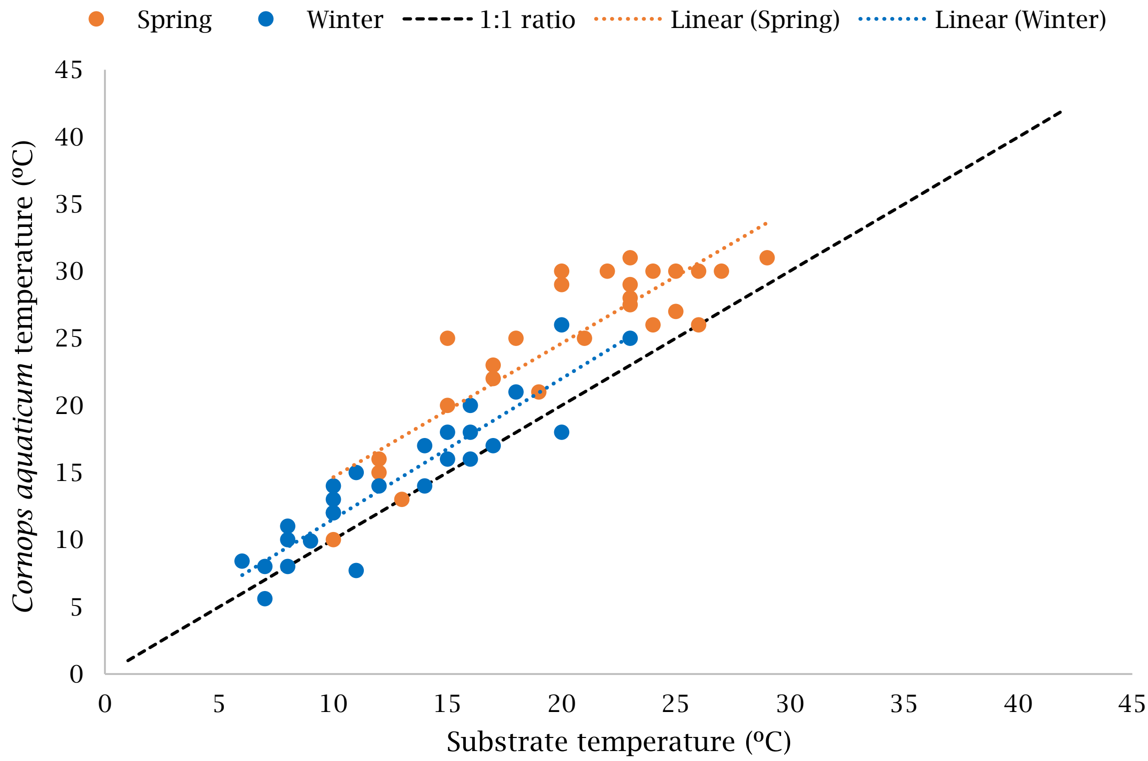


Figure 4.6. Relationship between substrate temperature (ºC) and *Cornops aquaticum* immatures body temperature (ºC) for spring (orange) ($y = 0.9982x + 4.67$) ($R^2 = 0.76$; $p < 0.001$) ($n = 26$) and winter (blue) ($y = 1.046x + 1.0935$; $p < 0.001$) ($R^2 = 0.85$) ($n = 26$). The dashed line depicts a 1:1 ratio between body temperature (ºC) and substrate temperature (ºC).

Discussion

This study investigated the effect of temperature on population numbers in a small outdoor population of *Cornops aquaticum* and measured the body temperatures of *C. aquaticum* adults and immatures during spring and winter conditions and their position in relation to their host plant. The results showed that the *C. aquaticum* population, underwent considerable mortality during winter, despite additions to the population during summer. Winter mortality of adults and immatures in 2017 and 2018 occurred over a longer period than expected, with numbers dropping consistently through May to July. This was in spite of daily canopy temperatures throughout winter still occurring within *C. aquaticum* individuals' behavioural range (CT_{min} of 6.29 ± 0.09 °C and 6.01 ± 0.08 °C for males and females respectively, Chapter 2). However, it should be noted that on particularly cold days canopy temperatures did drop below the behavioural range of *C. aquaticum* for at least two hours during the early morning. Acclimation is assumed to have occurred as temperatures were lower during a cold front (> 72 hours at 11.6 ± 3.2 °C) in June 2017 than the methodology used (72 hours at 13 ± 2 °C) in Chapter 2 for thermal acclimation testing. Compared to the thermal acclimation protocol, individuals in the outdoor population spent longer periods at conditions colder than their behavioural range (CT_{min}) and experienced colder temperatures preceding the cold front. This likely caused similar, if not greater, thermal acclimation physiological responses to those noted in Chapter 2. However, the increased overall thermal stress may also have resulted in higher mortality (Einum et al., 2019; Rohr et al., 2019). This could have been balanced by the greater thermal variability in the outdoor conditions, which may have resulted in thermoregulatory compensatory responses, such as basking, and thus diminish the physiological acclimation (Markle and Kozak, 2018).

The overall mortality in *C. aquaticum* could have been attributed to several components including climatic conditions, predation, disease or senescence (Chapman and Page, 1979). Given the lifespan of *C. aquaticum* adults (90 - 120 days), the senescence of grasshoppers likely impacted the population over the course of the experiment (Adis and Junk, 2003). However, this is unlikely to cause the drops in population numbers noted but likely did have a consistent effect throughout the 18 months. The experimental design excluded predation, as far as possible, by caging grasshoppers to exclude predators and removing predators from the water hyacinth plants. Although predators were actively removed, numerous spiders were still found in amongst the water hyacinth leaves. As such, if predation did impact mortality significantly, the mortality rates during summer were not nearly as severe as those noted during winter. Thus, the response to predation may be strongly linked to temperature with winter temperatures potentially reducing predator avoidance strategies (Marvin et al., 2016). The brown colouration noted in the native population likely has implications to their thermal biology during the winter months, assisting in basking and improving their ability to camouflage, thus reducing their predation risk. *Cornops aquaticum* adult behavioural responses to predators were similar to those described of the adults observed in their native range (Silveira-Guido and Perkins, 1975; Ferreira and Vasconcellos-Neto, 2001).

Mortality based on the effect of declining plant quality was likely less than field sites as water hyacinth plants were changed regularly. Thus, declining plant quality during winter may be a significant factor further hampering *C. aquaticum* population survival through winter, preventing establishment. The outdoor cage population density was high, especially after additions were made in December 2017 which has been shown to raise predatory and disease-driven mortality rates (McCauley et al., 2011). However, based on the regular removal of predators and the fine meshed cage, the overall predation rates are likely considerably lower than those experienced at water hyacinth field sites. Disease may have the affected survival of immatures more strongly, especially at higher densities and this may explain the mortality noted during February 2018 and April 2018 (Kistner and Belovsky, 2014).

Based on the average canopy temperature and the known $CT_{min,}$ of *C. aquaticum,* the likelihood of temperature directly causing the mortality is low as mortality was consistently high irrespective of how close the canopy temperatures were to the CT_{min}. The low temperatures however could have caused thermal stress to the grasshoppers, decreasing their overall condition and reducing their ability to forage effectively, resist disease or escape predators (Sinclair et al., 2003; Kingsolver and Woods, 2016; Wojda et al., 2017; Lubawy et al., 2019).

During spring and summer, adult grasshoppers appear to maintain body temperatures well above the substrate temperature. This preferred range may be linked to the fecundity of the *C. aquaticum* females in the same way as the pygmy grasshopper, *Tetrix subulata* (Orthoptera: Tetrigidae). Whereby females improved oviposition rates and the time to lay their first clutch in warmer temperatures (Forsman, 2001). This temperature selection may be particularly important to South African *C. aquaticum* females as, due to their smaller size, they may not have the same capability as larger females to deposit large clutches of eggs (Adis et al., 2008; Berger et al., 2008; Parsons and Joern, 2014). The similarity of *C. aquaticum* adult body temperature and substrate temperature during colder conditions may highlight a lack of effective thermoregulation. The comparable substrate and body temperatures of immatures during both spring and winter draws attention to the likely need for immatures to thermoregulate through behaviours such as basking in both summer and winter (Inglis et al., 1996; Onsager, 2000; Samietz et al., 2005;

Whitman, 2008). A lack of effective thermoregulation can affect predator avoidance and nutritional efficiency (Barton, 2011; Clissold et al., 2013). This may highlight mortality of immature *C. aquaticum* as a key factor limiting the overall adult population (Chapman and Page, 1979; Hovenkamp, 1989; Kistner and Belovsky, 2014; Despland, 2018).

Water hyacinth as a habitat/substrate for *C. aquaticum* offers a selection of microhabitats during spring and summer. However, during colder winter conditions, it may warm more slowly than the surrounding vegetation. The higher occurrence of *C. aquaticum* at the base of the plant or off the plant during winter could highlight two different thermoregulation strategies being employed, whereby the movement off the plant may be linked to *C. aquaticum* searching for new microhabitats to use as thermal refuge. This does correspond well with the fact that *C. aquaticum* adults were unable to maintain their preferred body temperature during winter and in their native range seek refuge in the sedge, *Oxycaryum cubense* (Poales: Cyperaceae) (Franceschini et al., 2011a). The vegetation structure, namely its vertical position, abundance, height and high leaf area index (LAI) protects grasshoppers from temperature extremes and wind speed (Edwards and Wratten, 1981). Of the many sedges indigenous to South Africa, it is unknown if any could provide a suitable microhabitat refuge to *C. aquaticum* (Cilliers et al., 1998; Gordon-Gray et al., 2009). This movement off the plant may however increase the risk of predation as *C. aquaticum* is known to use the architecture and colouration of water hyacinth to hide from predators (Silveira-Guido and Perkins, 1975; Ferreira and Vasconcellos-Neto, 2001).

Grasshoppers of other species are known to select new microhabitats based on vegetation structure and different substrate characteristics, for camouflage (Otte and Joern, 1975; Joern, 1982; Willott, 1997). Movements to different habitat patches may also be linked to the body condition of *C. aquaticum* and to the local abundance of water hyacinth or its quality, which deteriorates during winter (Parker, 1984; Owens and Madsen, 1995; Byrne et al., 2010). Body condition has been shown to be important in dispersal events with dispersers in good condition more likely to be successful (Stamps, 2006; Baines et al., 2015).

Species mobility between habitat patches may be important as a populational response to habitat change, whether it is seasonal or in response to events such as flooding (Ferreira and Vasconcellos-Neto, 2001; Adis and Junk, 2003). Grasshopper species of the Cape Floristic Region, with the greatest mobility were more likely to become widespread in the face of habitat modification (Adu-Acheampong and Samways, 2019). Therefore, the movement off the plant may be an indication of selection for other plant species, different water bodies or better climatic conditions.

Interestingly, the immatures did not seem to move off the host plant nearly as much as the adults. The sluggish behaviour noted here at colder temperatures (15 °C - 20 °C) is likely due to the fact that the metabolic rates of immatures were severely depressed at 15 °C to 25 °C (Chapter 3) and may explain why basking on the upper leaves is more common but as the upper leaf surface is most exposed this likely carries an increased risk to predation (Barton, 2010). This reduced activity may be the result of quarantine conditions where populations were consistently warm, daily temperature fluctuations were mild and temperature extremes were eliminated. This likely resulted in selection for smaller individuals that could increase their body temperature quickly in warmer conditions, and develop faster overall as colder conditions were mild and rare (Zuo et al., 2012). Similar selection of the thermal tolerances was observed in *Drosophila melanogaster*, which in response to more constant rearing temperatures had decreased cold and heat tolerance and longer recovery time from chill coma (Condon et al., 2015). The behavioural response of *C. aquaticum* immatures to predators seems to rely on camouflage and a static posture. A similar behavioural response was noted in immatures of the lubber grasshopper where frog predation increased as they became more mobile (Hatle and Grimké Faragher, 1998). The type of predator has been shown to affect habitat selection by grasshoppers, with grasshopper assemblages selecting upper vegetation when protected from birds (Pitt, 1999). The selection of the upper leaves may also be linked to the fact that birds generally feed on grasshoppers that are larger than 500 mg (Belovsky and Slade, 1993) or that spider induced mortality is higher in instars (Oedekoven and Joern, 1998; Joern et al., 2006). Therefore, instars may be more conspicuous to predators like birds but they are more likely to escape predators like spiders that occur on the lower leaf surfaces (Pitt, 1999).

It should be noted that extinction of the outdoor population did not occur. This suggests that "renaturalising" could be possible and the traits for survival/persistence at lower temperatures may still be present in the South African population. However, attempts at "renaturalisation" will likely require consistent releases to the field sites to prevent local extinction in a manner similar to more augmentative biological control strategies (Hajek et al., 2016;

Amadou et al., 2019) as was done in this study. The potential damage *C. aquaticum* populations may inflict of water hyacinth make it a good candidate for augmentative biological control programmes.

Chapter 5

The effect of temperature on feeding rates, development, oviposition rates and survival of *Cornops aquaticum*

Introduction

A better understanding of the life-history characteristics (LHCs) of biological control agents and their population dynamics is fundamental to help in establishing a sufficiently large population size in order to bring about a new population equilibrium in their target plant (Center et al., 2005; Plouvier and Wajnberg, 2018). Quantification of the potential biomass removed by biological control agents is similarly important in order to estimate loss of competitive advantage in their target plant and thus control solutions (Doyle et al., 2002). Climatic conditions, particularly temperature, are commonly investigated in such cases due to their strong influence on potential biomass removal and population dynamics through changes in species feeding activity and life-history characteristics (LHCs) such as development, fecundity and survival (Grafton-Cardwell et al., 2013; Aboubakar Souna et al., 2017; Damos et al., 2018).

The water hyacinth grasshopper *Cornops aquaticum* (Orthoptera: Acrididae) was released in South Africa in January 2011 (Bownes and King, 2010; Bownes et al., 2011) to control the invasive water hyacinth, *Pontederia crassipes* (Commelinales: Pontederiaceae). The highly damaging nature of *C.*

aquaticum in its native range suggested it would be a good addition to the existing biological control efforts of water hyacinth in South Africa (Hill et al., 1999; Bownes and King, 2010; Franceschini et al., 2013), however successful establishment of *C. aquaticum* has not been observed.

The main mediators of interactions between plants and their biological control agents are plant quality and temperature (Stiling and Cornelissen, 2007; Lee and Roh, 2010). This interaction for biological control is fundamental as the invasive alien plant (IAP) must support a healthy population of biological control agents and the competitive ability of the IAP must be reduced. Plants respond to damage in different ways; by increasing their growth or increasing the production of feeding deterrents or a combination (Bentz et al., 1995; Mardani-Talaee et al., 2016). For example, improved plant quality can positively or negatively affect biological control agent performance depending on the IAP (Sauge et al., 2010). Higher nitrogen concentrations in plants can also result in altered secondary plant phenolic compound production which can deter feeding (Hakulinen et al., 1995; Shan et al., 2018). Higher nutrient content including nitrogen and phosphorus, have been linked to insect LHCs such as survival, fecundity and feeding damage (Awmack and Leather, 2002; Lavoie and Oberhauser, 2004). Specifically, available nitrogen is a limiting factor to insect growth with increased plant nitrogen availability generally associated with improved performance (Kaul, 1977, Mattson, 1980; Wheeler, 2003).

Nitrogen fertilization improved the survival, fecundity and development of the biological control agent, *Pareuchaetes insulate* (Lepidoptera: Arctiidae), whilst increasing the height, stem diameter and leaf length of the IAP, *Chromolaena odorata* (Asterales: Asteraceae) (Uyi et al., 2015). However, it is thought that as the optimal level of nitrogen for the biological control agents is reached, the improved performance of the agents may be reduced as the level of secondary defensive chemical deterrents in their respective IAPs increases (Mardani-Talaee et al., 2016). In addition, the plant response to damage by the biological control agent, by either increasing growth rates or increasing the secondary metabolites, can be equally as important to understand the level of damage occurring (Schonhof et al., 2007; AlJabr et al., 2017; Ramazanova et al., 2019).

Whether plant compensation can accommodate for the damage occurring and how this compensation changes with different temperature regimes or plant quality is an important consideration in the efficacy of biological control agents (Chabot and Hicks, 1982; Lindroth et al., 1997). This has been the case for water hyacinth, where eutrophic water bodies severely hamper biological control efforts (Hill and Coetzee, 2017). Thus, the manner in which a plant responds is an important consideration to understanding the impact feeding has and the potential for control of an IAP. At a population level, plant quality can have a positive effect on fecundity and population growth cycles of insects which in turn affects future plant damage (Denno and Fagan, 2003).

The other mediator of plant-insect interactions, temperature, has been shown to impact consumption rates by insects and mediate the effect of plant quality. Higher ambient temperatures have been linked to increased plant consumption by insects (DeLoach and Cordo, 1976; Ferro et al., 1985; Lactin and Johnson, 1995; Chikwenhere, 2000), which is most likely associated with an increased metabolic rate (Wigglesworth, 1974; Forno and Bourne, 1985; Samways and Wilson, 1988; Stiling and Cornelissen, 2007). The effect of temperature on insects has been proposed to provide the necessary means by which insects can fully optimize higher plant quality, probably through faster digestion, better assimilation or increased general activity (Lindroth et al., 1997; Lee and Roh, 2010).

Higher ambient temperatures may also assist an insects' ability to cope with low plant quality, as compensation for low plant quality is generally accomplished through increased consumption and/or improved digestibility and assimilation rates in the insect (Mattson, 1980; Scriber and Slansky, 1981). For instance, individuals of the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae), consuming low protein high carbohydrate diets compensate by feeding more frequently than individuals feeding on high protein and high carbohydrate diets (Simpson and Abisgold, 1985). The compensation of *Samea multiplicalis* (Lepidoptera: Crambidae) larvae to low nitrogen foliage resulted in increased frass production, likely the result of increased consumption (Wheeler and Halpern, 1999). Compensation seems to be affected by the temperatures the species are exposed to. The migratory locust seems to maximize mass gain by selecting high temperatures to increase feeding rates at the cost of less efficient macronutrient to body mass conversion rates (Miller et al., 2009). Quantification of insect damage at different temperatures may complement the current understanding of the role of nutrients in insect herbivory, particularly *C. aquaticum* (Bownes et al., 2013a, 2013b).

The measurements of developmental rates usually assume a curvilinear relationship between temperature and insect development based on the fact that thermal performance curves are nonlinear (Colinet et al., 2015; McCalla et al., 2019). However, linear developmental models still constitute a large portion of models based on their adequacy over a broad range of temperatures and knowledge of their limitations (Shi et al., 2011; Damos and Savopoulou-Soultani, 2012; Quinn, 2017). Degree-day models are useful to make estimations of development in order to understand and predict population dynamics (Kingsolver, 1989; Stahl et al., 2019). These models assume a constant increase in development rate as temperature increases with a minimum developmental threshold (t), below which development does not occur. The accumulation of a certain number of degree-days is necessary for different developmental stages such as egg hatching or insect moulting to occur (Roltsch et al., 1999). The limitations include not accounting for reductions of development rates as a thermal maximum is reached and potentially overestimating or underestimating the developmental rate depending on fluctuating temperatures (Worner, 1992). Similar to development rate, temperature has been strongly linked to fecundity and mortality (Zhou et al., 2010). Both high and low temperatures can have significant impact on the egg production and survival of life stages of different biological control agents (Aboubakar Souna et al., 2017; Barbosa et al., 2019).

Previous studies on *C. aquaticum* have shown that higher water nutrient levels had a positive effect on female body mass, fecundity, survival but negatively affected *C. aquaticum* developmental rates (Bownes, 2009; Bownes, 2013a). In addition, temperature and photoperiod have been found to be very important for the development in *C. aquaticum* in its native range (Capello et al., 2007). Water hyacinth is a fast growing IAP and the addition of agents needs to reduce its biomass accumulation considerably. *Cornops aquaticum* has been shown to have a large herbivory effect on water hyacinth, if their population densities are high enough (Bownes et al., 2010a). However, compensatory growth by water hyacinth has been shown, even under low nutrient levels, in both simulated herbivory trials and in response to damage by the water hyacinth weevil (Soti and Volin, 2010; Mukarugwiro et al., 2018). Therefore, whether the grasshopper can have a negative effect on water hyacinth, especially at a density that is more similar to what is noted in the native range (one grasshopper per plant) has not been sufficiently investigated. The degree of compensation has also not been noted at different temperatures to assess whether the impact of herbivory may change seasonally and at different sites.

Female grasshoppers appear to consume water hyacinth at a rate twice that of males, removal of 0.127 ± 0.051 g/day and 0.060 ± 0.025 g/day for females and males respectively (Capello et al., 2011). Although overall biomass removal is an important characteristic it should be noted that instars cause abrasions to the leaves while adults are more likely to cause greater damage through feeding holes (Bezemer et al., 2003; Martinez and Franceschini, 2018). A sex ratio of 1:1 is noted in the native range and thus an accurate feeding damage estimate should attempt to match this (Bownes et al., 2010a; Capello et al., 2011).

The quantification of *C. aquaticum* herbivory on water hyacinth in South Africa is required to understand the likelihood of effective control. In addition, the effect of different temperatures on development, reproductive potential and survival is key to understanding population dynamics and why establishment difficulties have occurred. This should show how the feeding intensity may change at different release sites or between seasons. Measuring grasshopper mass gain at different temperatures in conjunction with frass production, may give an indication of survival and potential attempts at compensation by *C. aquaticum*.

The main aim of this chapter was to measure the effect of temperature on *C. aquaticum* feeding and LHCs, such as oviposition and development. The objectives were to measure the effect of different temperatures on 1) feeding damage and how the plant responds to this feeding damage, 2) oviposition rates in adults, 3) the development and survival proportion of eggs and instars, and 4) to calculate the developmental threshold and thermal constant of *C. aquaticum*.

Materials and Methods

Culture

Water hyacinth was grown in pools at the University of the Witwatersrand. Water hyacinth was grown in a high nutrient solution for eight weeks prior to use in the experiment. The nutrient solution simulated hypertrophic conditions found in most South African water bodies with NO_3^- (nitrate) at 7 mg.L^{-1} and $(PO_4)^{3-}$(phosphate) at 0.9 mg.L^{-1} (Wonder lawn and leaf; 7:1:3; Holmes, 1996; Byrne et al., 2010). Water nutrient levels were tested weekly using a Hach colorimeter DR/870. The concentration of nitrogen and phosphorous were maintained at 7 mg/L (nitrogen) and 1 mg/L (phosphorus), which has been shown to be close to optimal conditions for water hyacinth (Reddy et al., 1989; Ripley et al., 2006). Chelated iron was also added every two weeks at a rate of 1 g.L^{-1} to allow optimal growth (del Mar Delgado, 1994).

Cornops aquaticum individuals used in these experiments were a combination of individuals from the University of the Witwatersrand Insectary and Quarantine Facility (Chapter 2), individuals from the Agricultural Research Council (ARC) as well as individuals from the South African Sugarcane Research Institute (SASRI).

Experimental design and protocol

Feeding experiments

Feeding was investigated at different temperatures using individual leaves and whole plants to draw attention to the possible biomass removal and the implications when considering more realistic plant-insect interactions. Fifty water hyacinth plant leaves (second, third, fourth, fifth and sixth) were removed from their petioles and had their surface area measured. The surface areas of the leaves were measured using a high-resolution photograph, taken at a fixed distance with a Nikon camera (Coolpix AW100) and calculated using the programme ImageJ (Version 1.48, NIH, USA: Schneider et al., 2012). These leaves were placed separately into 50 petri dishes each with a grasshopper of either sex who had been starved for two hours. The petri dishes were separated into the temperature-controlled rooms set at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C (12:12 LD; 10 petri dishes per treatment). These were left for 24 hours, after which the leaf surface areas were remeasured and the difference calculated. Twenty water hyacinth leaves had their surface area measured and were dried in the oven (60 °C) for 24 hours. These were weighed in order to make surface area measurements to dry mass removal conversions for leaves fed on by *C. aquaticum*.

One hundred and forty-four water hyacinth plants were rinsed and had their ramets removed before being placed into 3 L plastic containers (two plants per container) filled with 2.5 L of tap water. Containers were then divided between three temperature-controlled rooms set at 15 ºC, 25 ºC and 35 ºC (12:12 LD). The surface areas of the leaves were measured using a high-resolution photograph, taken at a fixed distance with a Nikon camera (Coolpix AW100) and calculated using the programme ImageJ (Version 1.48, NIH, USA; Schneider et al. 2012). The third, fourth and fifth leaves of water hyacinth were prioritized based on the reported feeding preferences by *C. aquaticum* in their native range (Penfound and Earl, 1948) and what was observed by Bownes (2009) in a South African laboratory culture, however all other leaves with feeding damage were also included. At each temperature, twenty-four plants were designated to act as controls while the other twenty-four plants were subjected to herbivory by *C. aquaticum*. Containers were covered with fine polypropylene frost bags to prevent individuals from escaping (1m high x 1.4 m radius). *Cornops aquaticum* individuals removed from the University of the Witwatersrand Insectary and Quarantine Facility culture kept in separate plastic containers (30 cm x 20.5 cm x 21.5 cm) for two hours at 25 ºC after which their mass was measured using a semi-micro Shimadzu balance (Libror AEG-45SM). This was to ensure excess frass would be eliminated and the feeding history of *C. aquaticum* individuals would have a minimal effect on feeding rates.

Two adults (1:1 sex ratio) were placed into the containers described above containing water hyacinth plants, and left for one hour to adjust to the conditions in the temperature rooms. This simulated the field grasshopper density and the natural sex ratio observed in their native range (Silveira-Guido and Perkins, 1975). Room, water and canopy temperatures in each room were measured using Thermochron® iButton® devices (DS1921G) during the experiment. Individuals were left to feed for five days before being removed and reweighed. Frass was collected from the bags covering the containers and from all leaves. The frass was thereafter weighed using a semi-micro Shimadzu balance (Libror AEG-45SM). Each leaf which showed signs of feeding damage was labelled, removed and had its leaf surface area measured.

Fecundity and oviposition rate

The overall fecundity of *C. aquaticum* was measured over the course of 105 days, by placing 20 freshly moulted and weighed adults (1:1 sex ratio) into a single 45 L plastic container (kept as described in Chapter 2). Temperature and humidity inside the insectary during this period ranged between 25 ºC and 33 ºC, while the humidity was 50 ± 10 %. Water hyacinth plants were replaced weekly with specific attempts to provide water hyacinth with sufficiently thick petioles (diameter > 2 cm) to allow egg deposition to occur (Hill and Oberholzer 2000; Oberholzer and Hill, 2001). The removed plants were partially dissected to access the petioles and the total number of egg packets and eggs oviposited for that week were recorded. The effect of temperature on oviposition was measured by placing two *Cornops aquaticum* adults (1:1 sex ratio per container) into 15 separate 10 L plastic containers with four to five water hyacinth plants. These were placed into temperature-controlled rooms set at 15 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC (three containers per treatment). This experiment was run until female death occurred with males being replaced until death of the female.

Egg and instar development

Fifty *Cornops aquaticum* adults were placed into separate 25 L plastic container (1:1 sex ratio) covered with a fine mesh with eight water hyacinth plants for five days (monitored daily). Initially eggs were partially dissected out and the number of eggs were counted, sterilized using a 2 % bleach solution and placed into 2 L plastic containers filled with moist paper towel to maintain a high relative humidity. This was found to have high rates of egg mortality (80 %) due mainly to mycosis (fungal infection) and desiccation. This was remedied by removing the whole plant once exploratory feeding holes in the petiole were noted, an indication of egg laying. These were dated and placed into 45 L plastic container and transferred to constant temperature rooms set at 10 ºC, 12 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC and 37 ºC. The 10 ºC and 37 ºC were to test temperatures where egg development was assumed to not occur. The rooms were monitored daily for hatching and egg mortality. Egg mortality was calculated by counting the number of unhatched eggs still present in the egg pod after hatching had occurred, in addition to exploratory holes in the petiole which became infected and thus no eggs hatched.

Once hatched, first instars were placed individually into 5 L plastic buckets with fresh water hyacinth plants covered with a fine mesh. Buckets were transferred to one of the temperature-controlled rooms (15 ˚C, 20 ˚C, 25 ˚C, 30 ˚C and 35 ˚C; 12:12 LD) and checked daily for moulting. The discovery of exuviae was used to indicate that moulting had occurred. Second instars were marked on their pronotum with yellow enamel paint using a toothpick and moved to a 45 L bucket with 10 - 12 plants. Upon daily inspection, if the marking was not noted the individual was assumed to have moulted and moved into a new 45 L bucket with 10 - 12 plants. This was repeated for third, fourth and fifth instars. Once fifth instars moulted, grasshoppers were sexed using a dissecting light microscope (Leica Wild M3B Stereo). Painting of the first instar was not done due to their overall size and fragility. The mortality and time taken to moult was recorded for each instar. Two power failures of the cooling system occurred in the 30 ˚C temperature-controlled room, and as a result no results were obtained for instar development at 30 ˚C.

Data analyses

To calculate leaf surface area images of water hyacinth leaves were transformed to binary images and the Triangle threshold algorithm was applied using the programme ImageJ (Version 1.48, NIH, USA; Schneider et al. 2012). Leaf surface area was calculated on control leaves and leaves exposed to herbivory. A one-way analysis of variance (ANOVA) was used to ascertain the significance of differences between the different temperature treatments for leaf surface area removal and mass loss/gain in *C. aquaticum* in the feeding experiment.

All data were tested for normality and transformed (log x+1) where necessary. A one-way ANOVA was used to compare the mean number of days required to hatch at the different temperatures, the overall developmental time between different instars and the reduction in surface area at different temperatures. A Tukey HSD post-hoc test was performed to highlight where significant differences between groups lay. The lower developmental threshold (t) and thermal constant (K) were calculated using the major axis regression, outlined in Ikemoto and Takai (2000). An alpha of 0.05 was used to denote significant differences between different rearing temperatures. The reduced major axis regression (Equation 2) was utilized for the calculation of instar developmental thresholds and thermal constants as described in (Ikemoto and Takai, 2000; Damos and Savopoulou-Soultani, 2012).

Results

Plant-insect interactions

Temperature significantly increased feeding rates on individual leaves in *C. aquaticum,* with higher temperatures resulting in greater reductions in surface area removed ($F_{4,45} = 13.61$; $p < 0.001$; Figure 5.1). Significant differences were not noted between 15 ºC and 20 ºC ($F_{4,45} = 13.61$; $p = 0.98$), 25 ºC and 30 ºC ($F_{4,45} = 13.61$; $p = 0.06$) and 30 ºC and 35 ºC ($F_{4,45} = 13.61$; $p = 0.95$). Based on dry measurements of water hyacinth average leaf dry biomass was 0.95 ± 0.09 g (mean ± SD). This corresponds to water hyacinth reductions mean (± SD) in g/grasshopper/day of 0.059 (± 0.028), 0.068 (± 0.04), 0.09 (± 0.036), 0.139 (± 0.037) and 0.150 (± 0.038) at 15 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC respectively.

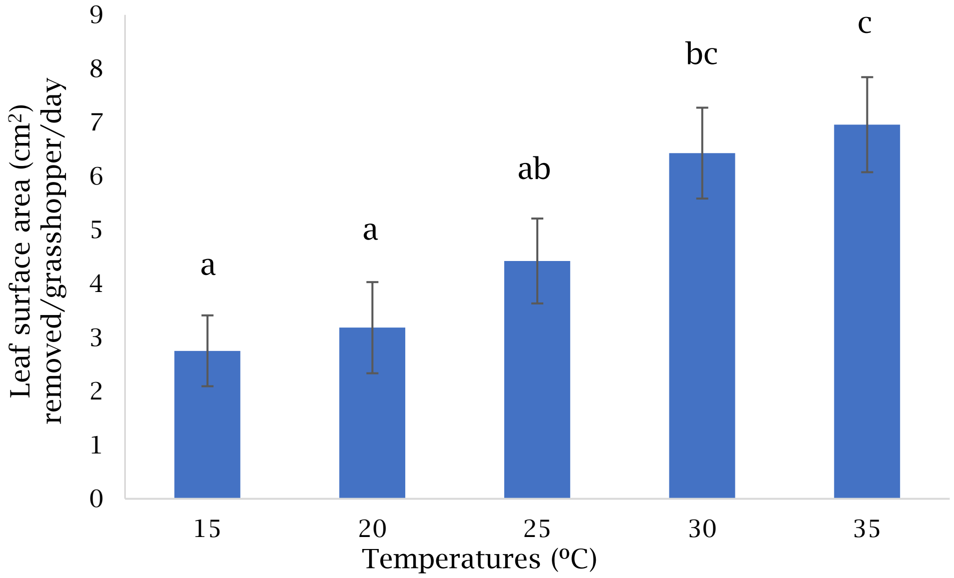


Figure 5.1. Average (mean ± SD; n = 50) reduction in leaf surface area (cm^2) by adult *Cornops aquaticum* per day at different temperatures for individual leaf measurements. Different letters denote significantly different means ($p < 0.05$).

Whole plants fed on by *C. aquaticum* had significantly smaller leaf surface areas than control plants at 25 °C ($t = 2.02$; $df = 42$; $p = 0.038$; Figure 5.2). There was no significant difference between the control leaves and leaves exposed to herbivory at 15 °C ($t = 0.73$; $df = 46$; $p = 0.47$) and 35 °C ($t = 1.29$; $df = 46$; $p = 0.20$). Leaf surface area of the leaves exposed to herbivory was found to be significantly different in the three different temperature treatments ($F_{2,69} = 4.12$; $p = 0.02$). No significant difference was found amongst the water hyacinth plants before the experiment commenced ($F_{5,138} = 2.28$; $df = 138$; $p = 0.98$). The reduction of surface area at 25 °C was 1.25 cm^2/day/grasshopper or approximately 0.06 ± 0.04 g/day/grasshopper.

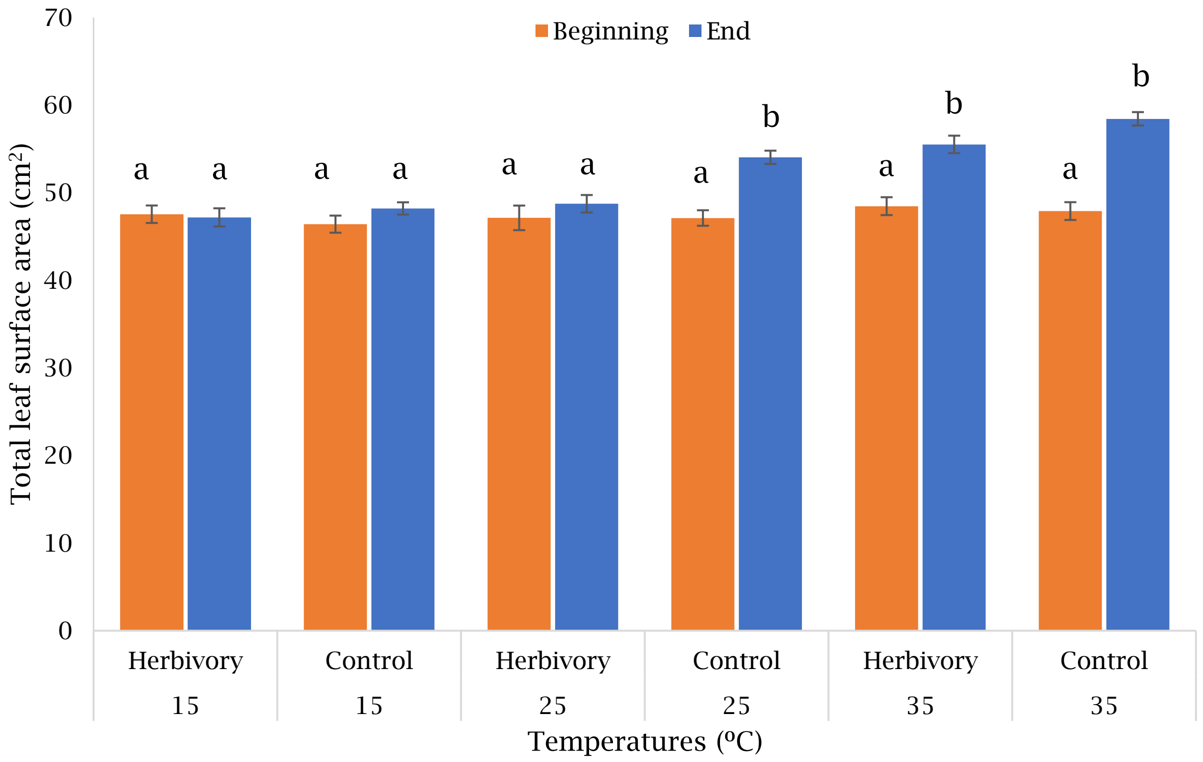


Figure 5.2. Mean ± SE of water hyacinth leaf surface area (cm^2) of the third, fourth and fifth leaves combined, either exposed to herbivory by *Cornops aquaticum* or unfed on control at 15 °C, 25 °C and 35 °C for whole plant measurements. Different letters denote significant differences between means ($p < 0.05$) ($n = 126$).

Despite the third, fourth and fifth leaves being the leaves commonly fed on, damage was also noted on the second and sixth leaves. *Cornops aquaticum* leaf area removal on the second and sixth leaves ranged from 5 % to 20 % of the leaf surface area and was only observed in the 25 °C and 35 °C treatments. The percentage difference between all control leaves and leaves exposed to herbivory was 6.21 %, 24.17 % and 14.54 % at 15 °C, 25 °C and 35 °C respectively. Feeding holes were more prominent on all leaves at 35 °C and on the fourth leaf at 25 °C (Table 5.1).

Table 5.1. Mean percentage reduction ± SE (%) in total leaf surface area (third, fourth and fifth leaves) caused by *Cornops aquaticum* induced feeding holes at 15 ºC, 25 ºC and 35 ºC.

Temperature treatment	Leaf number		
	Third	Fourth	Fifth
15 ºC	1.09 ± 0.35	0.13 ± 0.01	0.01 ± 0.02
25 ºC	0.22 ± 0.11	5.28 ± 3.92	0.95 ± 0.93
35 ºC	5.40 ± 1.88	8.47 ± 3.58	6.44 ± 3.18

Change in body mass by *C. aquaticum* was significantly higher at 35 ºC than at 25 ºC and 15 ºC (Figure 5.3; F = 8.555; df = 26; p = 0.002). Frass production increased dramatically with increased temperature, with wet frass mass totalling 11.41 g, 34.19 g and 106.73 g at the 15 ºC, 25 ºC and 35 ºC treatments respectively.

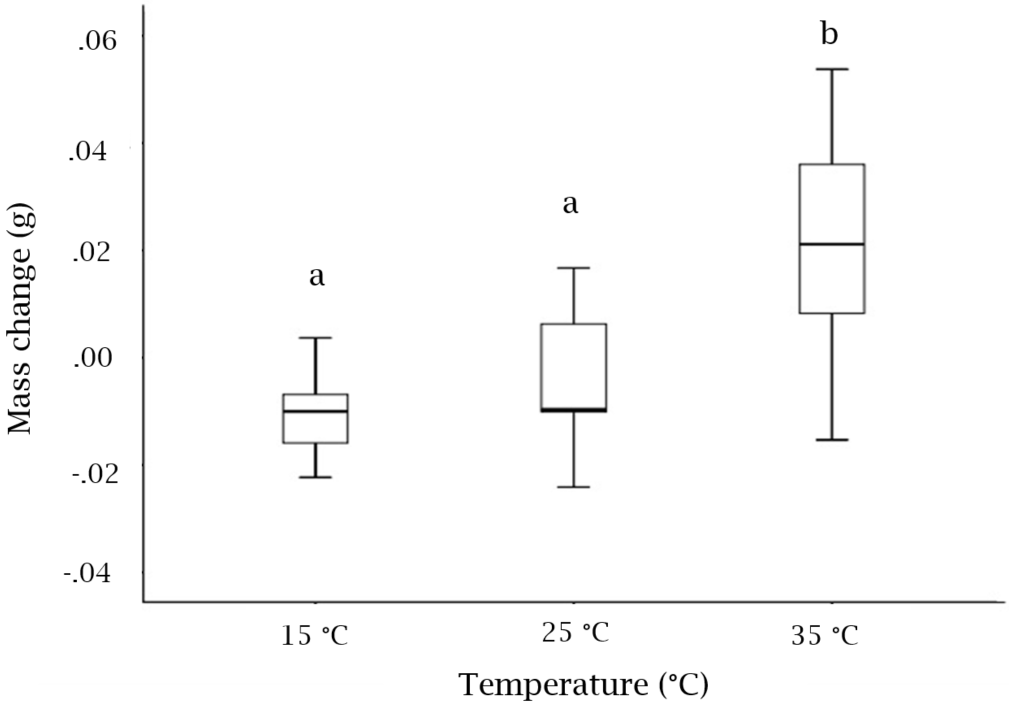


Figure 5.3. Mass change of adult *Cornops aquaticum* over five days at 15 ºC, 25 ºC and 35 ºC indicating the medians, range and interquartile range. Different letters denote significant differences ($p < 0.05$) (n = 46).

Egg and instar development

Temperature was found to have significantly affected egg hatching with warmer temperatures resulting in faster development ($F_{3,77}$ = 445.28; $p < 0.001$; Table 5.2). Of the five instar stages, development duration was the shortest in the first instar as temperatures increased ($F_{3,34}$ = 55.75; $p < 0.001$). Developmental time in the second instar was significantly slower ($F_{3,27}$ = 50.54; $p < 0.001$) at 15 °C, but development at 25 °C was not found to differ significantly from 20 °C ($F_{3,27}$ = 50.54; $p = 0.37$). Development for the second instar was fastest at 35 °C ($F_{3,27}$ = 50.54; $p = 0.04$).

Developmental time of the third instar was significantly different at all temperatures tested ($F_{3,31}$ = 194.7; $p < 0.001$) however no instars survived beyond this stage in the temperature-controlled room at 15 °C. Developmental time in the fourth instar did not differ significantly at 25 °C and 35 °C ($F_{2,31}$ = 13.2; $p = 0.07$) but both were significantly faster than at 20 °C ($F_{2,31}$ = 13.2; $p < 0.001$). The fifth instar was also found to have significantly different developmental times at all temperatures ($F_{2,23}$ = 34.7; $p < 0.001$). The fifth instar had significantly longer developmental duration compared to other instar regardless of the temperature ($F_{4,155}$ = 14.04; $p < 0.001$).

A strong relationship was noted between temperature and the developmental time of eggs and instars however this relationship became weaker in the fourth and fifth instars (Table 5.3). The developmental threshold of eggs was found to be 8 °C while the overall thermal constant was 981.13. The sex ratio of the fifth instars at 20 °C, 25 °C and 35 °C were 1:2.6, 1:1.43 and 1:0.66 (male:female) respectively.

Table 5.2. Development duration (mean ± SE) for *Cornops aquaticum* eggs and instars at different temperatures. Different letters denote significant differences between temperatures ($p < 0.05$).

	Developmental duration (days)					
		Instar				
Temperature	Egg	First	Second	Third	Fourth	Fifth
15 °C	52.67 ± 2.39^{a}	14.17 ± 0.33^{a}	14.67 ± 1.05^{a}	15.75 ± 0.75^{a}	-	-
20 °C	27.67 ± 0.88^{b}	9.75 ± 0.25^{b}	8.17 ± 0.75^{b}	11.25 ± 0.35^{b}	13.4 ± 0.94^{a}	15.25 ± 0.71^{a}
25 °C	23 ± 0.21^{c}	7.5 ± 0.26^{c}	6.95 ± 0.23^{b}	6.96 ± 0.19^{c}	7.38 ± 0.45^{b}	12.36 ± 0.31^{b}
30 °C	19.11 ± 0.17^{d}	-	-	-	-	-
35 °C	24	5.9 ± 0.21^{d}	6.06 ± 0.14^{c}	6.13 ± 0.20^{d}	6.86 ± 0.52^{b}	10.89 ± 0.33^{c}

Table 5.3. Mean ± SE lower developmental thresholds (t) and mean ± SE thermal constants (K) for *Cornops aquaticum* eggs and instars using the reduced major axis method.

Developmental stage	Developmental threshold (t)	Thermal constant (K)	Regression	
			R^2	p value
Egg	8.02 ± 0.62	378.1 ± 17.68	0.817	<0.0001
1	5.95 ± 0.54	135.7 ± 4.50	0.752	<0.0001
2	9.82 ± 1.00	99.5 ± 8.53	0.697	<0.0001
3	11.73 ± 1.50	95.72 ± 11.61	0.464	<0.0001
4	9.45 ± 1.33	108.63 ± 10.04	0.36	<0.0001
5	11.81 ± 1.90	163.48 ± 27.15	0.404	<0.0001
All	9.46	981.13		

Survival

Temperature had a considerable impact on the survival of eggs with temperatures of 20 ºC and below having mortality greater than 69 % (Figure 5.4). Even at more favourable conditions, such as 25 ºC, mortality was still high (58 %). No development was noted at the 10 ºC and 37 ºC treatments. A significant effect of temperature on the survival of instars to adulthood was noted with temperatures of 20 ºC resulting in only 10 % overall survival while none survived to adulthood at 15 ºC (Figure 5.5).

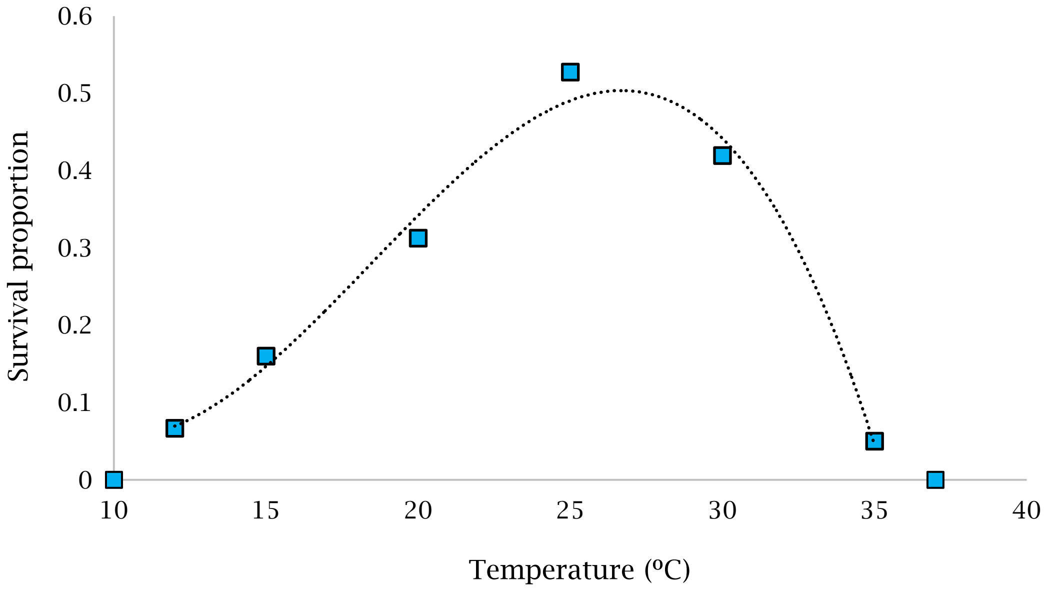


Figure 5.4. The effect of temperature on *Cornops aquaticum* egg survival. The dotted line shows a third order polynomial trendline ($y = -0.0002x^3 + 0.0111x^2 - 0.1637x + 0.7836$) ($R^2 = 0.9846$) where no zeros were included in the calculation (n = 203).

The total number of eggs produced at the beginning, middle and end of a females' life did not vary significantly ($F_{2,33} = 3.29$; $p = 0.55$). The average number of egg packets oviposited per female was 4.00 ± 0.87 (mean ± SD; standard error = 0.29; n = 9; Figure 5.6). Egg packets had an average of 29.55 ± 14.20 eggs (mean ± SD; standard error = 2.41; n = 32). The body mass of freshly moulted adult *C. aquaticum* females was 0.2822 ± 0.063 g (mean ± SD; n = 9). High oviposition rates were noted at temperatures above 25 ºC, with no oviposition was noted at temperatures at or below 10 ºC (Figure 5.7).

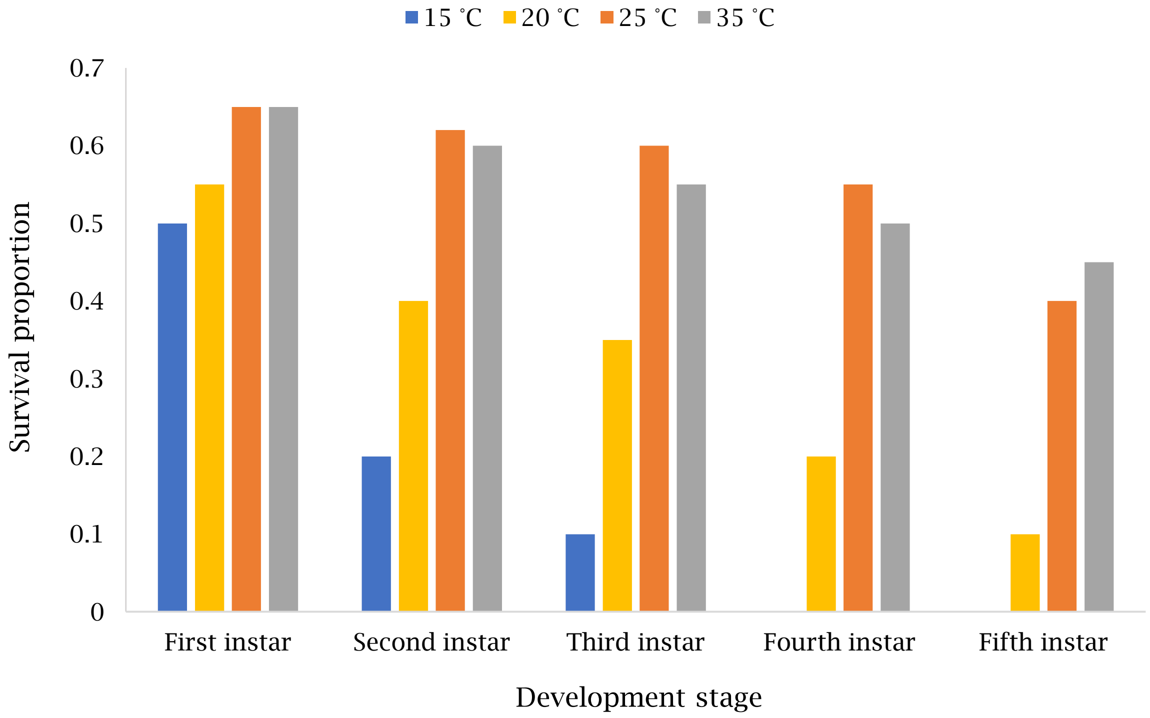


Figure 5.5. *Cornops aquaticum* instar survival at each instar stage at 15 ºC, 20 ºC, 25 ºC and 35 ºC.

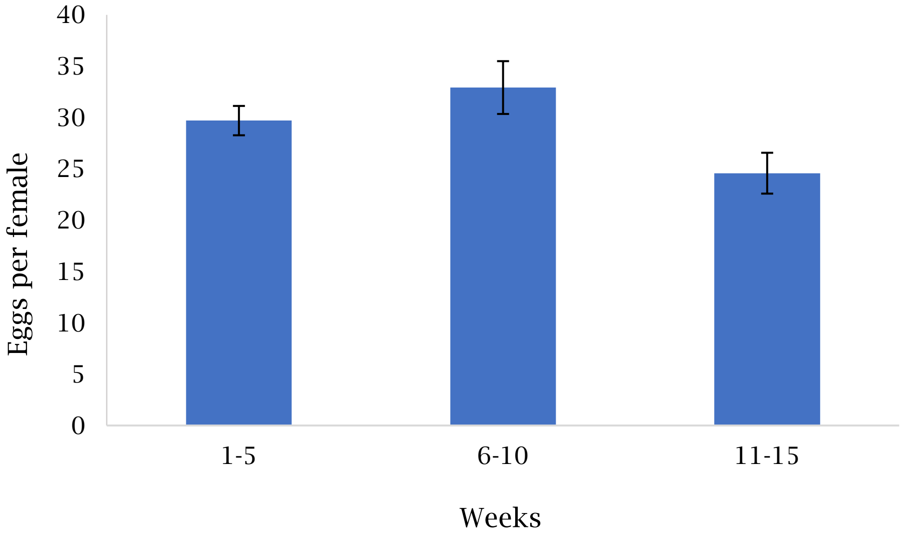


Figure 5.6. Number of eggs oviposited by *Cornops aquaticum* females (n = 9) into the petioles of water hyacinth over a period of 105 days at 25 - 33 ºC and 50 ± 10 % humidity.

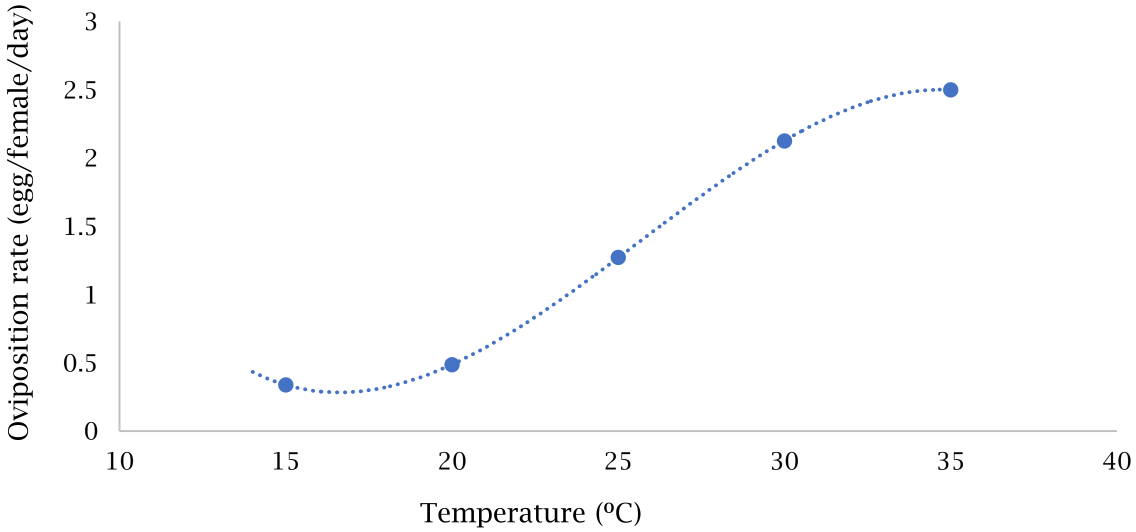


Figure 5.7. Oviposition rate (egg/female/day) of *Cornops aquaticum* at 15 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC (n = 75). The dotted line denotes a third order polynomial regression ($y = -0.0007x^3 + 0.0573x^2 - 1.2872x + 9.2654$) ($R^2 = 1$).

Discussion

Effect of temperature on feeding

The results show the importance of temperature as a mediating factor in insect feeding rates, plant growth and insect LHCs. Leaf surface area removal by *C. aquaticum* was strongly influenced by temperature with temperatures over 30 °C maximising feeding rates on leaves. At temperatures of 25 °C removal rates were found to be similar to measurements in the native range, with Capello et al. (2011) showing removal of 0.127 ± 0.051 g/day and 0.060 ± 0.025 g/day for females and males respectively. These measures also fall within the broader range shown by Franceschini et al. (2011b) with reductions of 0.029 to 0.119 g/grasshopper/day. At 30 °C and above however, the reduction of water hyacinth biomass observed (0.136 ± 0.037 g/grasshopper/day) was greater than daily removal rates measured in its native range (Capello et al., 2011; Franceschini et al., 2011b). The difference in biomass removal is likely a result of the constantly high temperatures and the removal of predation in the experiment, as daily temperature fluctuations and predation are known to negatively affect feeding, even at favourable average temperatures (Thaler et al., 2012). Plants were of a high quality and thus likely caused reduced feeding damage, suggesting *C. aquaticum* may have a greater damage potential than shown here (Levesque et al., 2002; Bownes et al., 2013b; Mukarugwiro 2018).

The reduction in surface area at 25 °C between control plants and plants subjected to herbivory (1.06 cm^2 per day) is similar to the 10 % overall damage (four days; 10-15 weevils/plant) by adult *Neochetina* weevils (*Neochetina eichhoniae* (Coleoptera: Curculionidae) and *Neochetina bruchi* (Coleoptera:

Curculionidae); Buchanan, 2013) at approximately a tenth of the numbers. *Neochetina* weevils have been highlighted as key biological control agents contributing strongly to overall biomass reductions of water hyacinth and a key contributor in the successful control of water hyacinth in Lake Victoria (Wilson et al., 2007; Jones et al., 2018). The differences in feeding on leaves compared to feeding on whole plants by *C. aquaticum* highlighted the need for whole plant experiments, as this allows plants to respond to herbivory by producing secondary metabolites, reducing digestibility (Mardani-Talaee et al., 2016; AlJabr et al., 2017). The significant difference in the surface area between the control leaves and leaves fed on by *C. aquaticum* noted in the 25 ºC and not 35 ºC treatment does highlight the importance of temperature in regulating plant tolerance to herbivory (Strauss and Agrawal, 1999; Tiffin, 2000). This is likely due to the fact that in the 35 ºC treatment water temperatures approached those needed for optimal water hyacinth growth (François, 1970; Sato, 1988). The larger feeding holes present at 35 ºC suggests damage by *C. aquaticum* was considerable, but the plants overall photosynthetic rates were greater than at lower temperatures and thus was able to compensate. The compensation of the plant may also be linked to an increase in the production of secondary metabolites reducing the overall leaf digestibility and deterring feeding (Schonhof et al., 2007; Adeyemi et al., 2010; Ramazanova et al., 2019).

Body mass changes and frass production

The high frass production, as seen at 35 ºC, has been linked to poorer digestibility of the water hyacinth leaves (McCullough and Kulman, 1991). However, based on mass gains at 35 ºC, adult *C. aquaticum* appear to be able to compensate adequately with high feeding rates (McCullough and Kulman, 1991).

The much higher frass production at 35 ºC appears to corroborate with the damage noted on the cut leaves experiment at temperatures above 30 ºC. The feeding on the cut leaves showed damage at 35 ºC almost double that of those observed at 25 ºC. Increased frass production may be the effect of higher temperatures causing an overall increase in the digestive rates or higher temperatures causing higher demand for nitrogen (Miller et al., 2009; Lemoine and Shantz, 2016). Lower levels of nitrogen in the leaves are unlikely to be the cause as the plants were grown in a nutrient rich solution for over eight weeks and likely had nitrogen still present in the roots which can be translocated to the shoots (Leghari et al., 2016). The plants were kept in tap water for the duration of the experiment and during this time the plants could not take up any nitrogen. This is likely to only affect the first leaf which are not commonly fed on by *C. aquaticum* and would therefore not impact the results of this experiment.

The body mass increases in *C. aquaticum* observed at 35 ºC compared to 25 ºC indicates a potential improvement in survival and fecundity. The body mass of grasshoppers, especially females, has been linked with increased survival and fecundity (Adis et al., 2008; Garrad et al., 2016; Walczak et al., 2017). The loss of body mass at 15 ºC indicates a potential problem at most field sites, as even at warmer sites, winter average temperatures are 15 ºC or lower for extended periods of time (Byrne et al., 2010). This may help explain the mortality noted during winter in outdoor experiments (Chapter 4). The different feeding rates and body mass changes noted at different temperatures in this chapter correspond to metabolic rate measurements of *C. aquaticum*

(Chapter 3), where metabolic rates in adults were at their highest at 25 ºC and 35 ºC and were disproportionately lower at 15 ºC.

Biological control of water hyacinth

As temperatures rise, *C. aquaticum* appears to perform better with increased body mass and higher feeding rates. The warmer temperatures promoted rapid growth of the plants, effectively reducing *C. aquaticum* efficacy despite higher feeding rates (You et al., 2014). It should be noted that the temperatures used in this study are higher than would be expected in the field, but illustrate the pattern that high temperatures may favour both the biological control agent and the IAP. If South African water bodies were not eutrophic, this increased feeding may lead to reduced plant growth even at higher temperatures as N and P become limiting (Reddy et al., 1989; Coetzee and Hill, 2012; Strange et al., 2018). However, the current eutrophic condition and warmer temperatures present at many South Africa field sites, a higher density of *C. aquaticum* will likely be required to have a significant impact on the plant (Bownes et al., 2010a; Byrne et al., 2010). It should be noted that this experiment is an individual reaction between plant and grasshopper and a large enough population density of *C. aquaticum* may exert a more pronounced herbivory pressure capable of reducing water hyacinth infestations even at optimal conditions for the plant. Bownes et al. (2010b) showed that higher densities of *C. aquaticum* are capable of reducing the competitive advantage of water hyacinth.

Egg development and mortality

The development of *C. aquaticum* eggs at 25 ºC was 23 ± 0.2 days, which is similar to the 20 - 30 days observed by Oberholzer and Hill (2001) and is slightly faster at warmer temperatures. The lowest levels of egg mortality occurred at 25 ºC and 30 ºC, which correspond well to most canopy temperatures at South African water hyacinth field sites during spring and summer months (Byrne et al., 2010). The high egg mortality at temperatures below 20 ºC and above 35 ºC are likely due to the fact that these were suboptimal temperatures for egg development and have been shown to cause higher egg mortality (Wu et al., 2013).

The overall high egg mortality, even at optimal conditions, noted in this study was not observed in other South African studies (Oberholzer and Hill, 2001; Bownes, 2009). The laboratory estimates of egg survival from this study may reveal egg survival under ideal conditions and thus may not mirror those in water hyacinth field sites where frost, petiole senescence and contamination may further hinder egg survival and emergence. As such, egg development at 15 ºC is likely a laboratory remnant, and is unlikely to occur at the field sites. The overall egg mortality of *C. aquaticum* was moderately higher compared to other grasshopper species and the *Neochetina eichhorniae* weevil associated with water hyacinth at similar conditions, 65 - 80 % and 80 % respectively (Hao and Kang, 2004; Hauptfleisch, 2016). Further studies should investigate pathogens associated with egg packets and the feeding hole by *C. aquaticum* in the field (Hilker and Meiners, 2002; Whitman, 2008; Hilker and Fatouros, 2015).

Cornops aquaticum females appear to produce eggs that only develop within a narrow temperature range. This may just be due to the fact that water hyacinth acts as a good temperature buffer or may be related to the more consistent temperatures experienced in quarantine conditions. The selection, either due to the buffering capacity or the time in quarantine, may be associated with a trade-off in survival. In two different strains of *Eccritotarsus catarinensis* (Hemiptera: Miridae), a similar trade-off was noted, where reductions in fitness traits occurred with warmer constant temperatures (Ismail and Brooks, 2016; Ismail et al., 2017; Porter et al., 2019).

The overall number of egg packets (4.0 ± 0.9) and the number of eggs within the egg packets (29.6 ± 14.2) are similar to those of Bownes (2009) with a range of two to five egg packets and 14 - 21 eggs per packet, but lower than those of Hill and Oberholzer (2000) who reported up to seven egg packets and 30 - 70 eggs per packet. The lower observed number of egg packets and number of eggs per egg packet highlight a potential reduced fecundity of the species now compared to when it was first brought into South Africa. The lower fecundity may be linked to inbreeding of the population over 80 generations in quarantine (Bownes, 2009; Taylor et al., 2011) or the fact that females are smaller and thus oviposit less eggs (Adis et al., 2008; Berger et al., 2008; Parsons and Joern, 2014; Walczak et al., 2017). The water hyacinth plants used were grown in a high nutrient solution and simulated eutrophic water conditions, and likely improved the *C. aquaticum* fecundity (Heard and Winterton, 2000; Ismail et al., 2017). However, fecundity can vary substantially between different conditions and at different sites. For example, in the native range of *C. aquaticum*, Vieira and Santos (2003) reported only 13 ± 5 eggs per egg packet while Oberholzer and Hill (2001) observed between 50 and 560 offspring per female when the population was first brought into quarantine. This high degree of variation in fecundity is likely the result of plant choice, plant quality and intra-populational differences between individuals (Ferreira and Vasconcellos-Neto, 2001; Capello et al., 2011).

The developmental threshold (t) of eggs was 8 °C and was lower than other water hyacinth biological control agents such as *Neochetina eichhoniae* (11.95 °C), *Neochetina bruchi* (11.4 °C) and *Eccritotarsus catarinensis* (10.3 °C; King, 2011; Paterson et al., 2019). This may highlight the phenotypic plasticity of the species (Adis et al., 2008). It also suggests that temperature is not directly halting development in *C. aquaticum* instars. The difference in the developmental threshold between *C. aquaticum* and the other water hyacinth biological control agents may be the position on the plant that eggs are oviposited, *C. aquaticum* lay their eggs in the petiole away from the sun's heat. Compared to other biological control agents of water hyacinth, the thermal constant for all developmental stages was lower than N. eichhoniae (1313.7) but considerably higher than E. catarinensis (341.75) and Eccritotarsus eichhorniae (286.7; King, 2011; Paterson et al., 2019).

Instar development and mortality

The faster developmental rates observed in the first and second instar may be a result of their small size and reliance on higher ambient temperatures in order to develop effectively. This may be due to body mass affecting the rate of heating and cooling based on surface area to volume ratios (Stone and Wilmer, 1989; Chown and Gaston, 2010). In the field this may explain the increased likelihood of *C. aquaticum* instars to be found basking on the top of water hyacinth leaves (Inglis et al., 1996, 1997; Onsager, 2000; Samietz et al., 2005; Whitman, 2008; Chapter 4). At 25 °C developmental duration was similar to those shown in the high nutrient treatment in Bownes (2009) and more similar to conditions in the native range (Capello et al., 2011; Capello and Franceschini, 2014; Vieira and Santos, 2003). At 35 °C, *C. aquaticum* developed faster than native populations of *C. aquaticum* across all developmental stages (Capello et al., 2011; Franceschini et al., 2014). The fact that the fourth and fifth instar had a significantly longer developmental time than the first three instar stages whose variance was least explained by temperature suggests that other factors may limit development at these stages. *Cornops aquaticum* development has been shown to be affected by plant quality and photoperiod (Adis and Junk, 2003; Bownes, 2010a). The weaker relationship between temperature and development duration noted at the fourth and fifth instars may have been due to the larger size of these instars. Higher body mass may have helped maintain higher temperatures in relation to ambient temperatures and allowed faster development even at colder temperatures. Duration of development was similar in the fourth and fifth instar, to what has been noted in other populations of *C. aquaticum*, however, due to the smaller sample size and diminished effect of temperature on development at these instars, the developmental threshold and thermal constant at these stages may have some degree of error (Vieira and Santos, 2003; Capello et al., 2011; Capello and Franceschini, 2014).

Plant quality and photoperiod have both been shown to influence mortality of *C. aquaticum* (Adis et al., 2004; Zerbino et al., 2013; Nissinen et al., 2017). Higher plant quality was found to cause high mortality in developing males (Bownes, 2009), however it is unlikely that plant quality affected mortality to the degree observed given the consistent replenishment of plants through the study. The survival to adulthood was more similar to those tested on low plant quality in Bownes et al. (2011). The considerable female-biased sex ratio noted at 20 °C may be due to the differences in body size between males and females. However, grasshoppers were only sexed after their final moult and thus this pattern may be result of the experimental design. The overall higher mortality seen across all developmental stages, compared to other native and introduced populations of *C. aquaticum*, could at least be partially explained by the photoperiod selected (12:12 L:D). Mortality appears to be strongly affected by the photoperiod, particularly in the instar stage, with short days (8:16 L:D) causing considerably higher mortality (55 %) and high survival in 24 hr light cycles (>80 %) (Adis et al., 2004; Zerbino et al., 2013). Interestingly, Adis et al. (2004) showed increased mortality of *C. aquaticum* with increasing temperatures while in this study, elevated temperatures improved developmental rates and decreased mortality rates. Alternatively, the mortality and development of instars may also be partially a result of the consistency of the temperature rooms. Fluctuating temperatures compared to constant temperatures have been shown in some species to result in faster development (Worner, 1992; Milosavljević et al., 2019).

Implications for population growth

The oviposition rate and slow development of eggs at colder temperatures suggests egg deposition and hatching during winter is unlikely to occur. It also suggests adult survival is crucial through winter in order to promote new population growth when conditions improve in spring. However, this will likely cause a lag in the *C. aquaticum* population and may diminish the overall effect on water hyacinth during winter and through its rapid growth in spring (Edwards and Musil, 1975; Gopal, 1987; Byrne et al., 2010). The lag in *C. aquaticum* population growth in spring may be even further exaggerated by the fact that oviposition is not possible on newer plants with slender petioles (Hill and Oberholzer 2000; Oberholzer and Hill, 2001).

Chapter 6

A model of water hyacinth growth and *Cornops aquaticum* population dynamics

Introduction

Species' modelling has been an important component in the understanding of real world population dynamics. Models vary in their type, construction and complexity depending on their purpose (Priesmann et al., 2019). The most often used model types in pest management are niche models and process based mathematical models (Damos and Savopoulou-Soultani, 2010; Heger et al., 2013). Mathematical models can be used to investigate changes in population number as a function of time considering particular phenological events, such as insect emergence, to plan the optimal release strategy of natural enemies or pesticide application frequency and the potential emergence of resistance (Tang et al., 2010; Liu et al., 2011; Kharouba et al., 2018). These models can be particularly useful in predicting pest outbreaks in different environmental conditions as insect development tends to be closely associated with environmental conditions such as temperature (Wang et al., 2019). As a result, these have been used to better understand invasion and control protocols, particularly under different environmental factors (Pilkington and Hoddle, 2006; Heger et al., 2013, Branson, 2014).

Most insect developmental models are constructed using temperature dependent instar development, based on the accumulation of degree days or thermal units, as well as the addition of life-history characteristics (LHCs) such as mortality and fecundity (Herms, 2004; Damos and Savopoulou-Soultani, 2010). These LHCs are generally based on laboratory data of a species under differing controlled laboratory conditions (Jian et al., 2018). Such a model is then used to estimate the potential population abundance and dynamics, which are key components of how biological control agents function at a population level to control their target invasive alien plant (IAP; Strayer et al., 2006; Heger et al., 2013).

Biological control models are generally inherently complex as they shift from general models to a more specific model, focused on a particular species in a new habitat (Kriticos et al., 1999; Gramig et al., 2015). Thus, research on insect population modelling investigates development, egg laying, mortality and the longevity of each insect life stage (Nissinen et al., 2017; Skovgard and Nachman, 2017). Key factor analysis has been used in many studies to identify the main influential factors in species-specific population dynamics (Yamamura, 1999; Yukawa et al., 2016). Manipulations of these models assist in the understanding of the system in question and where improvements to the model can be made (Moore et al., 2012; Yukawa et al., 2016).

Many successful models for water hyacinth exist, each tailored to its own specific goal. These include the estimation of production of methane gas as well as in invasive plant control management (Lorber et al., 1984; Akbay et al., 1991; Wilson et al., 2001). Lorber et al. (1984) used multiple parameters such as solar radiation and nutrients, to closely replicate field data. Wilson et al. (2005) used a logistic model of water hyacinth growth largely developed from water temperature and nutrient concentration parameters calculated from the literature. Population growth rates can be more easily estimated in animals than plants, but in the case of aquatic plants, growth can be closely linked to water temperature (van der Heide et al., 2006). Despite the suite of possible factors affecting water hyacinth growth, South African water bodies and their eutrophic condition may be best simulated by differences in temperature between sites alone. As water hyacinth is limited in its population growth due to the physical capacity of water bodies, logistic growth models are particularly suited to this plant (Wilson et al., 2005). The logistic model simulates a system that will grow until a particular level is reached, which is determined by the environmental conditions. This is known as the carrying capacity of a system and is defined as the maximum number or biomass of a particular species that can occur in a particular habitat without diminishing the resources to limit future generations of the same species (Mäler, 2000; Acou et al., 2011).

Additions to these existing water hyacinth models have been made to try to simulate the impact herbivory, by natural enemies, could have on the control of water hyacinth. This was initially modelled using Lotka-Volterra equations to describe the relationship between plant and herbivore, but this had mixed success (Room, 1990; Wilson et al., 2001). A modification of Laissez-faire model (Caughley and Lawton, 1981) has been used recently to highlight the possible impact of herbivory on water hyacinth using water hyacinth weevils (Hauptfleisch, 2016). In the context of *Cornops aquaticum* (Orthoptera: Acrididae) where establishment has so far failed, models can allow a simulation of establishment, using environmental variables from water hyacinth field sites and the specific *C. aquaticum* developmental, fecundity and survival data derived from laboratory studies (Chapter 5). In addition, manipulation of specific parameters of this model may assist in the understanding of the sensitivity of specific life stages, which are usually the earlier stages which suffer higher mortality than other stages. In addition, these models can highlight important areas for further research. This may allow a better understanding of the establishment difficulties or highlight improved approaches to water hyacinth control with other potential biological control agents. To better understand the lack of establishment of *C. aquaticum* and its potential to control water hyacinth, the potential population dynamics must be further explored. The aim of this chapter was to 1) describe a temperature-dependent *C. aquaticum* model based on development and survival, 2) highlight possible population dynamics for instars and adult stages at different field sites over one year and 3) ascertain the potential biomass removal of water hyacinth by *C. aquaticum*.

Materials and methods

Model description

A model was constructed in the software STELLA 9.14 (Isee Systems, New Hampshire). In STELLA, populations and their dynamics are represented visually in a spatial map and then assigned mathematical functions based on empirical data as well as data from the literature. Specific STELLA tools are associated with particular functions based on the LHCs. For example, the “stock” tool (rectangle) operates like a container where quantities such as the population density can be altered (Figure 6.1). These changes are manipulated through “flow” tools, represented as valves with an arrow denoting the direction from a particular start point (cloud). If a flow is directed into a stock the change to the stock is positive and if the flow is directed away from a stock the change is negative. Connectors (pink arrows) denote connections between different model variables and elements (arrows can be straight or curved). Converters (circles) usually act as variables, which work alongside connectors and function in the modification of inflows and outflows. The conveyor tool (rectangle with vertical lines) works based on a transit time where a stock reservoir is maintained for only a predetermined time span, after which it is removed from the stock through an outflow (shown as removal in Figure 6.1). Populations in STELLA can be accounted for in a variety of ways depending on the needs of the user. Thus, for the purpose of a continuous daily model such as this one, where several aspects of a model are running simultaneously, most parameters in this model are viewed as arrays. Arrays, represented as layered/stacked stocks, conveyors and flows, provide a simple visual representation to show parallel model structures. The step size (dt) function was set to 1 to correspond to time in days. For example, calculations as general as the overall *C. aquaticum* population number and as specific as the number of hatched eggs are calculated daily. Further descriptions can be found in the STELLA 9.14 package (Isee Systems, 2006).

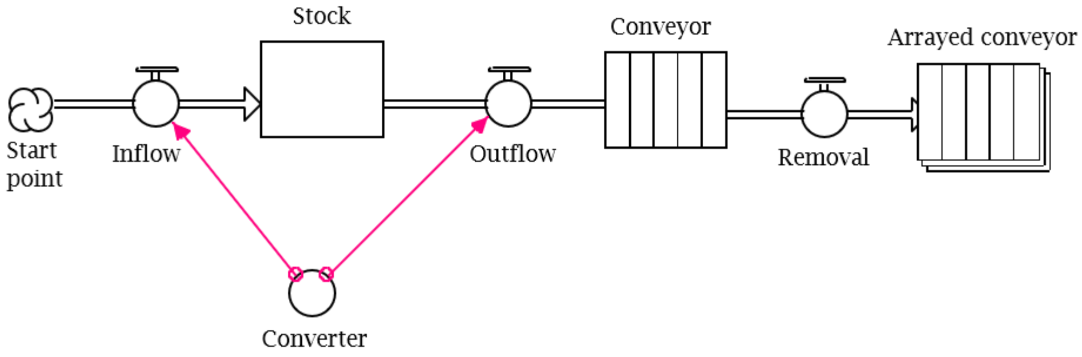


Figure 6.1. Diagram showing modelling tools of STELLA modelling software.

Model development

Water hyacinth

Biomass density is generally used to estimate the magnitude of water hyacinth infestations due to the size of the water bodies restricting growth and the fact that the bulk of the plant negatively impacts on growth (Wilson et al., 2001). For aquatic plants like water hyacinth, the area of the open water is assumed to be the maximum volume, while the biomass is generally limited by the amount of nutrients available (Lugo et al., 1978; Wilson et al., 2005). The carrying capacity of water hyacinth has been assumed to be 66000 g/m^2, as this was the maximum observed in the field under eutrophic conditions (Lugo et al., 1978; Wilson et al., 2005). The overall rate of change in the population (dB/dt) can be represented by the equation:

$$\frac{dB}{dt} = r_B . B(1 - \frac{B}{K}) \tag{1}$$

Where r_B is the intrinsic growth rate per day, B is the current population density (g/m^2) and K is the carrying capacity. The daily growth rate has been found to vary considerably, ranging from to 0.027 to 0.1 g/g/day (grams of growth per gram of water hyacinth per day) depending on the nutrient levels, water temperature and solar radiation (Madsen, 1993; Heard and Winteron, 2000; Methy et al., 1990; Sale et al., 1985; Kobayashi et al., 2008; Eid and Shaltout, 2017). For the high nutrient conditions and the average water temperatures of South African water bodies, an intrinsic daily growth of 0.06 g/g/day was assumed to account for fluctuations between seasons (Byrne et al., 2010; van Ginkel, 2011; Matthews and Bernard, 2015). Macrophyte growth has been shown to be closely linked to temperature wherein relative growth rate declines outside upper and lower temperature thresholds (van der Heide et al., 2006). Wilson et al. (2005) modelled the effect of temperature on water hyacinth growth and set the lower and upper temperature threshold as 8 ºC and 40 ºC, while the optimal temperature for growth was 30 ºC. These support estimates of growth rates in the field by Batanouny and Elfiky (1975) and Gopal (1987). Thus, the r_B can be altered to a relative growth rate using the third-order polynomial equation from van der Heide et al. (2006):

$$R(T) = cT_W(T_W - T_{min})(T_{max} - T_W) \tag{2}$$

Where R is the relative growth rate of the macrophyte population at a particular water temperature (T_w), c is the empirical scaling constant (8.7×10^{-6}) and the upper and lower thresholds for water hyacinth growth are T_{max} and T_{min} respectively (Figure 6.2). Although water hyacinth biomass, particularly at sites that experience frost, does decrease in winter months, it was assumed this would be minimal at warmer field sites where *C. aquaticum* would more likely be released (Byrne et al., 2010). Therefore, negative growth of water hyacinth was not incorporated into the logistic and temperature dependent model. The model was run using average daily water temperature data (provided by the ARC-PPRI; using Thermochron® iButton® devices (DS1921G) located 8 cm below water surface) from two water hyacinth sites; Farm Dam, Gauteng (25º26'08.2" S 31º39'21.6" E) and Golden Citrus, Mpumalanga (25º54'57.8" S 27º58'01.9" E). These provide a colder and warmer field site which represent the likely range of water hyacinth growth rates of South African water bodies. The model was run for a period of one year, with day one starting on the first of December.

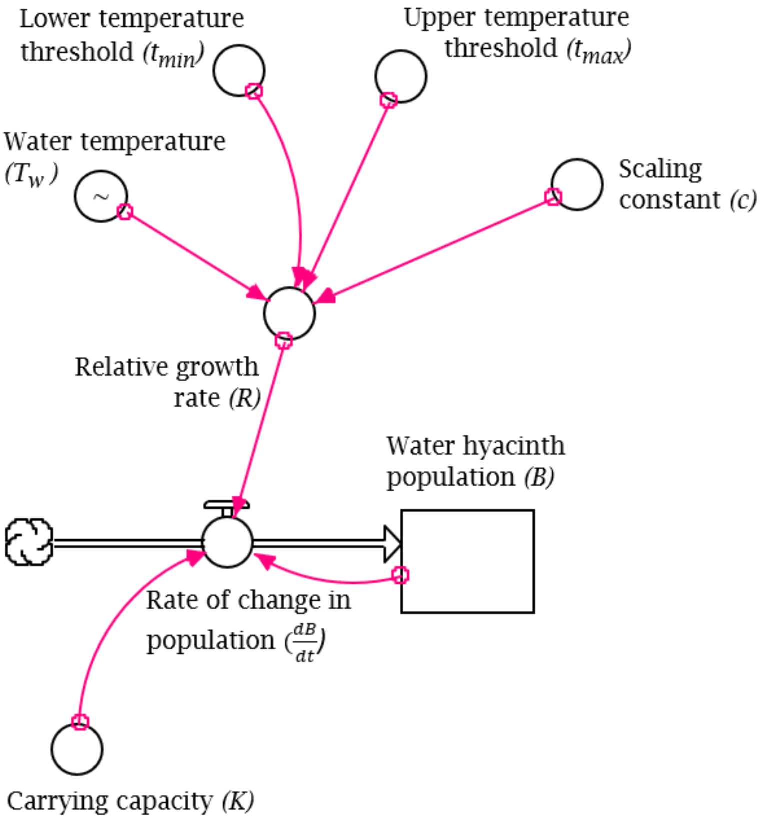


Figure 6.2. Logistic growth model of water hyacinth using a relative growth rate (*R*) based on water temperature (T_w) from van der Heide et al. (2006). The propositional logic/approximation symbol (~) on the water temperature converter signifies the variable varies with time.

Cornops aquaticum

Based on the LHCs of *Cornops aquaticum* (Chapter 5) the model was built using three sub-models to denote adult senescence, egg development and instar development. The adult senescence period used was 120 days, based on the maximum adult lifespan of *C. aquaticum* noted in the literature (Adis and Junk, 2003). Given the overall number of plants in a quadrat (m^2), and the abundance of grasshoppers in the native range of 6.8 ± 6.61/ m^2 individuals, a conservatively high carrying capacity of 21000 (7 adults/m^2) and 15000 (5 instars/m^2) was assumed to occur in a 3000 m^2 field site (Byrne et al., 2010;

Franceschini et al., 2011b). Once individuals made it to the adult stage it was assumed that they did not undergo any further mortality other than through senescence. The reproductive output was based on the temperature dependent oviposition results from Chapter 5. Both egg and instar development were modelled using degree day accumulation calculations from Chapter 5. The model was run using the water hyacinth average daily canopy temperature data (provided by the ARC-PPRI; using Thermochron® iButton® devices (DS1921G) located 11 cm above the water surface) from the monitoring sites Farm Dam and Golden Citrus. Golden Citrus provides a warmer water hyacinth site where *C. aquaticum* establishment is more likely and Farm Dam provides a colder site for comparison, based on mean canopy temperature for all the water hyacinth monitoring sites (Byrne et al., 2010). Model variables, their associated units and the source are listed in Appendix 1.

Array check

In order for the model to work in a daily sequential way an INIT and a COUNTER function were added as converters to the model using an IF-THEN-ELSE logic equation:

$$S_a[*] = if\ C_d = [*]\ then\ C/dt\ else\ 0 \tag{3}$$

Where $S_a[*]$ represents a particular array and C_d represents the count of the number of days the model has been running. This causes the model to only start a model component when a certain number of days have passed. For example, the array for day 4 will only initiate when the days passed in the model reaches 4.

Adult senescence sub-model

A conveyer function was used to represent the adult population where adults were held for a period of 120 days before senescence (*S*) removed them from the population (Figure 6.3). The key aspect is the transit time which denotes the lifespan of *C. aquaticum.* The number of adults at a particular time was represented by the equation:

$$A_t = A_{t-1} + (A_i - S)$$

(4)

Where (A_{t-1}) is the number of adults at the previous time step and changes occur based on adult inflow (A_i) and outflow through senescence (*S*). The inflow of adults (A_i) was defined as:

$$A_i = M_5 * M_S^5$$

(5)

Where adult inflow (A_i) is dependent on the product of the number of fifth instars moulting (M_5) and the surviving proportion of fifth instars (M_S^5).

An initial inflow of 1000 adults were made into the adult conveyor to simulate the initial release (I_r) of *C. aquaticum* on day 1. This was accomplished using the array check equation and an IF-THEN-ELSE logic function, such that:

$$I_r = IF\ S_a[*] > 0\ then\ 0\ else\ 1000$$

(6)

Where the adults are only added to the conveyor at time 0.

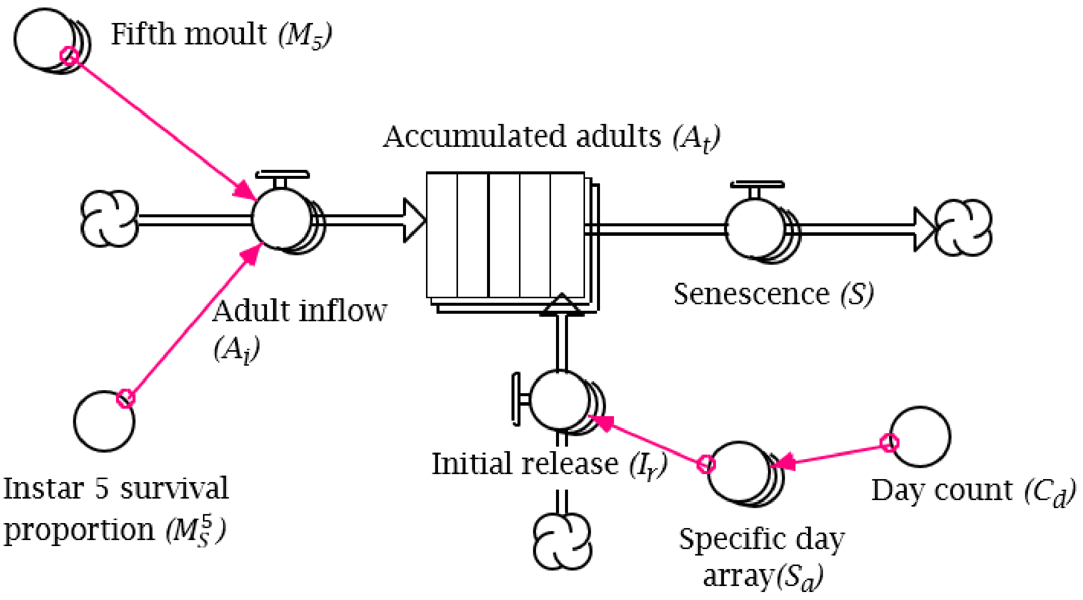


Figure 6.3. Adult senescence sub-model. Senescence (S) denotes adult longevity which was assumed to be 120 days, the maximum stated in the literature (Adis and Junk, 2003). Inflows are determined by the number of fifth instars moulting (M_5) and survival proportion of the fifth instar (M_S^5) in addition to an initial adult release (I_r) of 1000 adults. Layered conveyor, inflows, outflows and converters denote variables were arrayed. The converters, day count (C_d) and specific day array (S_a) were used to ensure specific arrays only ran on specific days.

Egg development sub-model

The accumulated eggs (E_t) at a particular time interval was represented by the difference between the inflow of new eggs being oviposited and the eggs hatching using the equation:

$$E_t = E_{t-1} + (E_i - E_h) \tag{7}$$

Where the system (E_t) is increased by eggs flowing into the system (E_i) and decreased by eggs hatching (E_h). The increase to the system can be described by:

$$E_i = O.A.0.5$$

(8)

The inflow of new eggs (E_i) is a product of the temperature driven oviposition rate (*O*) and the current number of *C. aquaticum* females (*A*), which is half the number of adults, assuming a 1:1 sex ratio. The oviposition rate was thus represented by the equation described in Chapter 5:

$$O = -0.0133C_t^2 + 1.1104C_t - 13.784$$

(9)

Where oviposition rate depends on the canopy temperature (C_t). The oviposition temperature threshold was incorporated into this oviposition rate equation using an IF-THEN-ELSE logic function, such that:

$$O = if\ (C_t \geq 10\ °C)\ then\ (-0.0133C_t^2 + 1.1104C_t - 13.784)\ else\ 0$$

(10)

Where oviposition is temperature dependent but only calculated when the canopy temperature (C_t) is greater than 10 °C (Chapter 5; Figure 6.4).

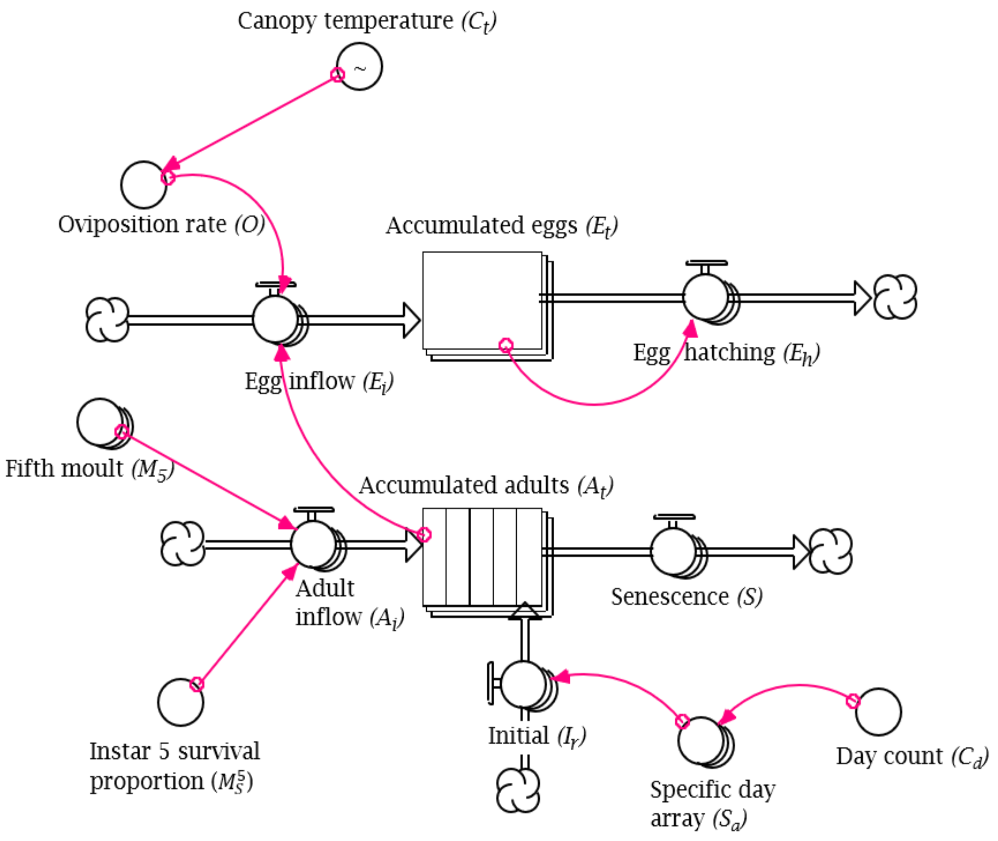


Figure 6.4. Accumulated egg sub-model. Accumulated eggs (E_t) are the difference between the inflow of new eggs being oviposited (E_i) and the hatching rate (E_h). The propositional logic/approximation symbol (~) present on the canopy temperature converter signifies the variable varies with time. Layered stocks, inflows and converters denote variables have been arrayed.

The hatching rate (E_h) was calculated using a cumulative degree day model where development through degree days was calculated with the equation:

$$°D = C_t - t \tag{11}$$

Where the daily canopy temperature (C_t) must be greater than the lower developmental threshold (t) for a degree days' development to occur.

The degree day (°D) represents the degree days accumulated over the course of a day. This used field canopy temperature data from two water hyacinth field sites and calculations of the lower developmental threshold from laboratory experiments on egg development in Chapter 5. The cumulative egg degree day model can be represented as the equation:

$$CDD_{e_t} = CDD_{(t-1)} + °D$$

(12)

Where CDD_{e_t} is the cumulative egg degree days at a particular time. As the CDD_{e_t} reached the egg thermal constant (K_e) the accumulated degree days were reset to zero. This was performed with an IF-THEN-ELSE logic function such that:

$$E_{h_t} = IF\ CDD_{e_t} \geq K_e\ then\ \frac{CDD_t}{dt}\ else\ 0$$

(13)

Where E_{h_t} is the eggs hatching at a specific time (Figure 6.5). The cumulative degree day model was then incorporated in the egg model (Figure 6.6).

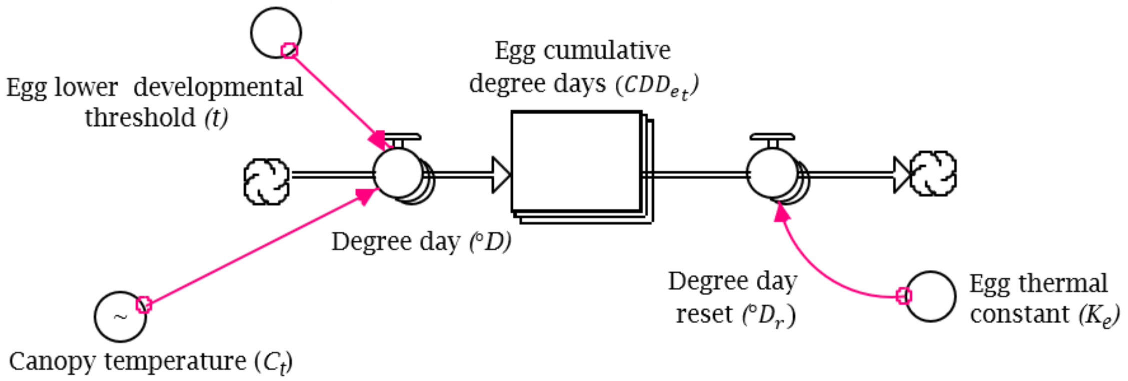


Figure 6.5. Egg cumulative degree day model. CDD_{e_t} represents the accumulated egg degree days based on the converters, canopy temperature (C_t) and the lower developmental threshold (t). The $°D$ denotes the degree days accrued over the course of the day while $°D_r$ denotes the reset function when the egg thermal constant (K_e) is reached. The converter K_e represents the thermal constant for the egg stage. Layered stocks, inflows and converters denote variables have been arrayed.

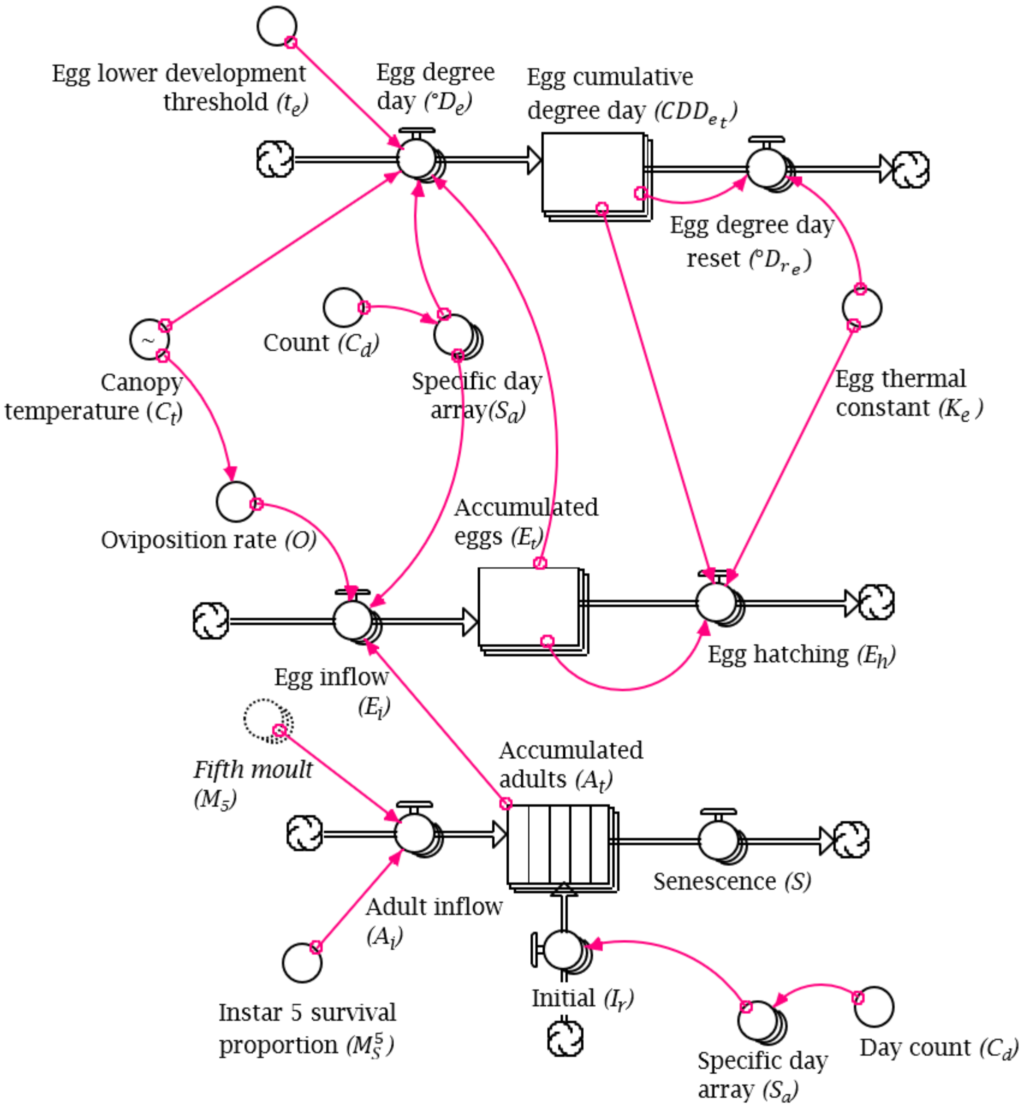


Figure 6.6. Combined cumulative degree day model and egg sub-model. Layered conveyor, inflows and converters denote variables have been arrayed.

Egg survival

Using the third order polynomial trendline from Chapter 5 the temperature dependent egg survival was represented by:

$$E_s = if\ C_t \geq 10\ then\ -0.0002C_t^3 + 0.0111C_t^2 - 0.1637C_t + 0.7836 \text{ else } 0$$

(14)

Instar development sub-model

As with the egg development sub-model, the instar development sub-model highlights dynamics in each instar stage based on the effect of temperature on development and survival. To reduce repetitiveness, the structure, description and parameterisation for only the first instar was represented diagrammatically. The accumulated number of instars at a particular time interval was represented by the differences between the inflow of new instars and those moulting as the outflow. The equation for the accumulated number of first instars (I_t^1) at a particular time was represented by the equation:

$$I_t^1 = I_{t-1}^1 + (I_i - M_1)$$

(15)

Where I_{t-1}^1 is the accumulated first instars at the previous time step and the system is increased by the inflow of new first instars (I_i) and decreased by first instars moulting (M_1). The accumulated number of second (I_t^1), third (I_t^1), fourth (I_t^1) and fifth (I_t^1) instars at a particular time was represented accordingly by:

$$I_t^2 = I_{t-1}^2 + (I_i^2 - M_2)$$

$$I_t^3 = I_{t-1}^3 + (I_i^3 - M_3)$$

$$I_t^4 = I_{t-1}^4 + (I_i^3 - M_4)$$

$$I_t^5 = I_{t-1}^5 + (I_i^4 - M_5)$$

(16-19)

The development of instars utilized the similar function to the egg model element where a cumulative degree day model was used to track development.

The first moult (M_1) was calculated using the cumulative degree day equation of the egg development (Equation 11), where development through added degree days was only calculated if the daily canopy temperature (C_t) exceed the lower developmental threshold (t). The cumulative first instar degree day model can be represented as the equation:

$$CDD_{i^1 t} = CDD_{(t-1)} + {}^{\circ}D \tag{20}$$

Where $CDD_{i^1 t}$ is the accumulated first instar degree days at a particular time. As the $CDD_{i^1 t}$ reached the thermal constant (K_i) and the first instar moulted, the accumulated degree days were reset to zero. This was performed with the IF-THEN-ELSE logic function such that:

$$M_1 = \ IF\ CDD_{i^1 t} \geq K_i\ then\ \frac{CDD_t}{dt}\ else\ 0 \tag{21}$$

Where M_1 is the number of instars moulting at a specific time. These equations were repeated for the second to fifth instars with parameters such as first moult (M_1) being changed accordingly to the second moult (M_2), third moult (M_3), fourth moult (M_4) and fifth moult (M_5).

Instar survival

Given the strong impact laboratory temperatures had on the early instar survival (Chapter 5) and metabolic rate (Chapter 3), it was assumed survival at field sites would be similar. The first and second instars survival was assumed to follow the similar survival curves to those seen in the temperature-controlled laboratory conditions (Chapter 5). Thus, the survival of the third instar (I_s^3), fourth instar (I_s^4) and fifth instar (I_s^5) was set at 0.85. The first (I_s^1) and second (I_s^2) instar survival was represented by the equations:

$$I_s^1 = if\ C_t \geq 10\ then\ -0.0002C_t^3 + 0.0111C_t^2 - 0.1637C_t + 0.7836 \text{ else } 0$$

$$I_s^2 = if\ C_t \geq 10\ then\ -0.0002C_t^3 + 0.0111C_t^2 - 0.1637C_t + 0.7836 \text{ else } 0$$

(22-23)

Where survival of first instar (I_s^1) and second instar (I_s^2) varied with canopy temperatures. The inflow for the instars was defined by the equations:

$$I_i^1 = E_h * E_s$$

$$I_i^2 = M_1 * M_S^1$$

$$I_i^3 = M_2 * M_S^2$$

$$I_i^4 = M_3 * M_S^3$$

$$I_i^5 = M_4 * M_S^4$$

(24-28)

Where instar inflows (I_i^1, I_i^2, I_i^3, I_i^4, I_i^5) are products of egg hatching (E_h), moults (M_1, M_2, M_3, M_4) and their respective survivals (E_s, M_s^1, M_s^2, M_s^3, M_s^4; Figure 6.7).

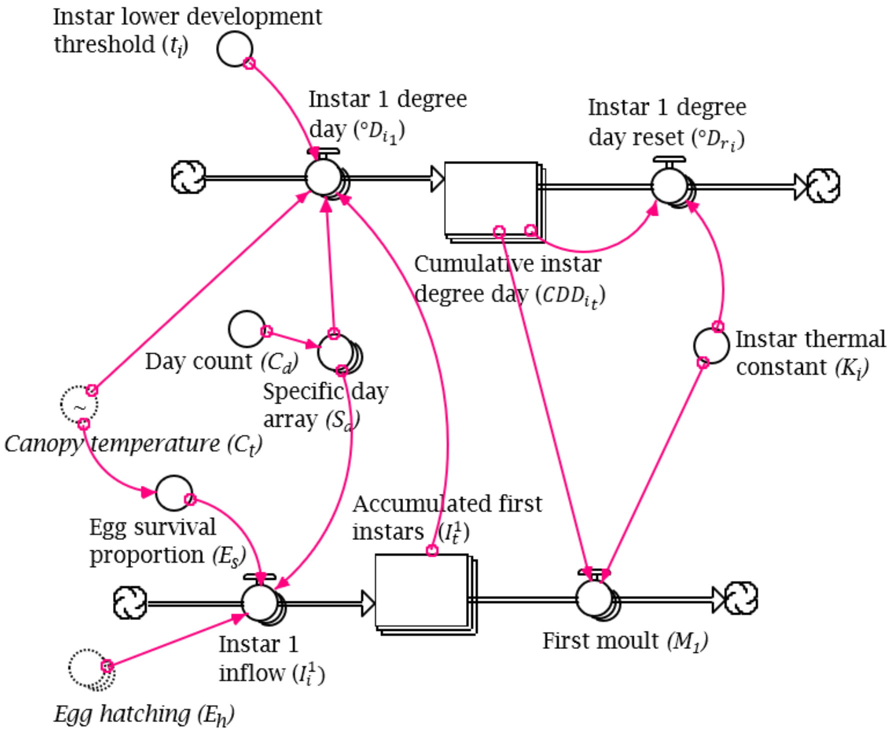


Figure 6.7. Combined degree day and instar development sub-models. Layered conveyor, inflows and converters denote variables have been arrayed.

Critical thermal minima, mortality and carrying capacity

Once chill coma occurs, an insect is unable to move or interact with its environment and thermal stress is accumulated. When the daily average canopy temperature was below the critical thermal minima of 6.15 °C (Chapter 2) it was assumed that most of the population would be in chill coma for at least 6 hours. As a result, a conservative 2 % mortality in each of the instar stages was assumed to occur per day due to a combination of accumulated thermal stress and overall vulnerability to predators. The instar mortality (D_c) was added to all accumulated instar (I^1, I^2, I^3, I^4, I^5) equations as an outflow and is represented by an IF-THEN-ELSE equation:

$$\mathrm{Dc} = IF\ C_t \le 6.15\ then\ (I_t^1 * 0.02)/dt\ else\ 0 \tag{29}$$

A sum of the arrays for adults and instars was used to incorporate the carrying capacity of adults and instars and monitor population numbers over time. This was represented by the equations:

$$A_T = \text{Arraysum}(\text{A}[*])$$

$$I_T^1 = \text{Arraysum}(I_t^1[*])$$

$$I_T^2 = \text{Arraysum}(I_t^2[*])$$

$$I_T^3 = \text{Arraysum}(I_t^3[*])$$

$$I_T^4 = \text{Arraysum}(I_t^4[*])$$

$$I_T^5 = \text{Arraysum}(I_t^4[*])$$

(30-35)

where A_T represents the total number of adults in the system at a particular time, summing all the specific arrays. Likewise, I_T^1, I_T^2, I_T^3, I_T^4, I_T^5 represent the total number of first, second, third, fourth and fifth instars in the system at a particular time. The total number of instars in the system (T_i) at a particular time was shown by the equation:

$$T_i = \text{Arraysum}(I_t^1[*]) + \text{Arraysum}(I_t^2[*]) + \text{Arraysum}(I_t^3[*]) + \text{Arraysum}(I_t^4[*]) + \text{Arraysum}(I_t^5[*])$$

(36)

The last component of the *C. aquaticum* model was the incorporation of instar and adult carrying capacity. This was done by modifying the array sum equation for total instars and adults such that:

$$A_T = \text{IF Arraysum(A[}*\text{])} < 21000 \; \mathit{then} \; \text{Arraysum(A[}*\text{])} \; \mathit{else} \; 21000$$

$$\text{T}_i = \text{IF T}_{i_t} < 15000 \; \mathit{then} \; \text{T}_{i_t} \; \mathit{else} \; 15000$$

(37-38)

Where the total adults (A_T) and total instars (T_i) could not exceed 21000 and 15000 individuals respectively (Figure 6.8).

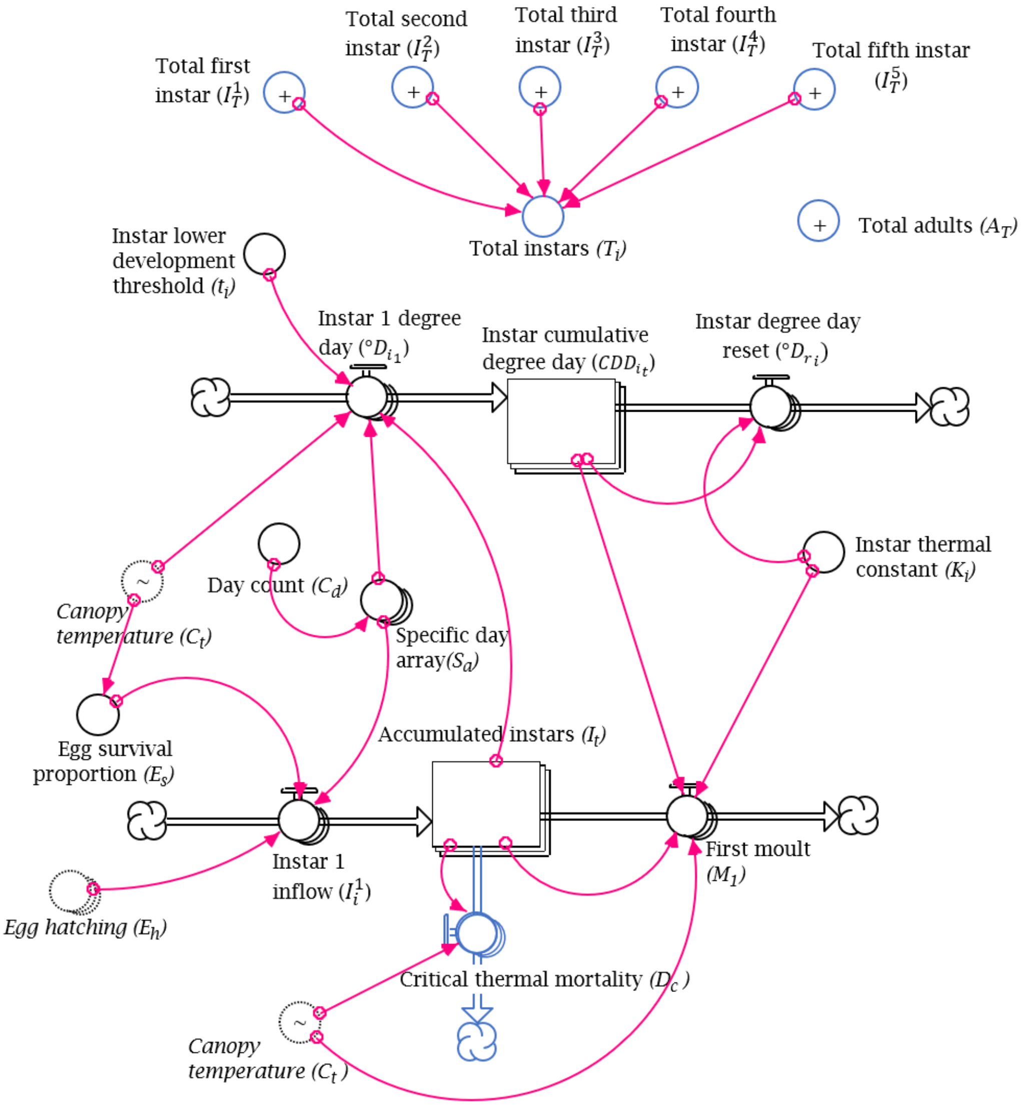


Figure 6.8. Combined degree day and instar sub-models accounting for instar mortality from prolonged exposure at or below the critical thermal minima. The sums of specific arrays are represented as converters with a plus symbol (+). Added model components are outlined in blue.

Biomass removal

The impact of *Cornops aquaticum* on water hyacinth biomass was investigated using the highest estimates of the daily biomass removed in the native range by *C. aquaticum* adults and instars, at 0.127 g/adult/day and 0.078 g/instar/day (Capello et al., 2011; Franceschini et al., 2011b). A basic functional response feeding model based on Caughley (1976) was used to simulate biomass removal by *C. aquaticum* adults and instars. The feeding damage (*F*) of the population of *C. aquaticum* instars and adults was based on the density of water hyacinth and the maximum rate of biomass removal per individual such that:

$$F = (c_1 A(1 - e^{-SB}) + (c_2 I(1 - e^{-SB})) \tag{39}$$

Where c_1 and c_2 are the maximum biomass removed for adults and instars respectively. The population numbers of adults and instars are represented by A and I, while B and S represents the density of water hyacinth biomass and the search efficiency of the herbivore. As *C. aquaticum* is a specialist grasshopper of the Pontederiaceae plant family and is found closely associated with its host, search efficiency was assumed to be one.

The maximum biomass removed for adults was estimated based on the temperature driven feeding rates described in Chapter 5 (Appendix 2). A third order polynomial regression equation was used to describe the relationship such that:

$$c_1 = -0.00003 * Ct^3 + 0.0026 * Ct^2 - 0.0602 * Ct + 0.4846 \tag{40}$$

The water hyacinth population density equation (dB/dt) was modified to:

$$\frac{dB}{dt} = \frac{dB}{dt-1} + r_b . B\left(1 - \frac{B}{K}\right) - F \tag{41}$$

Where feeding damage (F) decreases the biomass of water hyacinth (Figure 6.9).

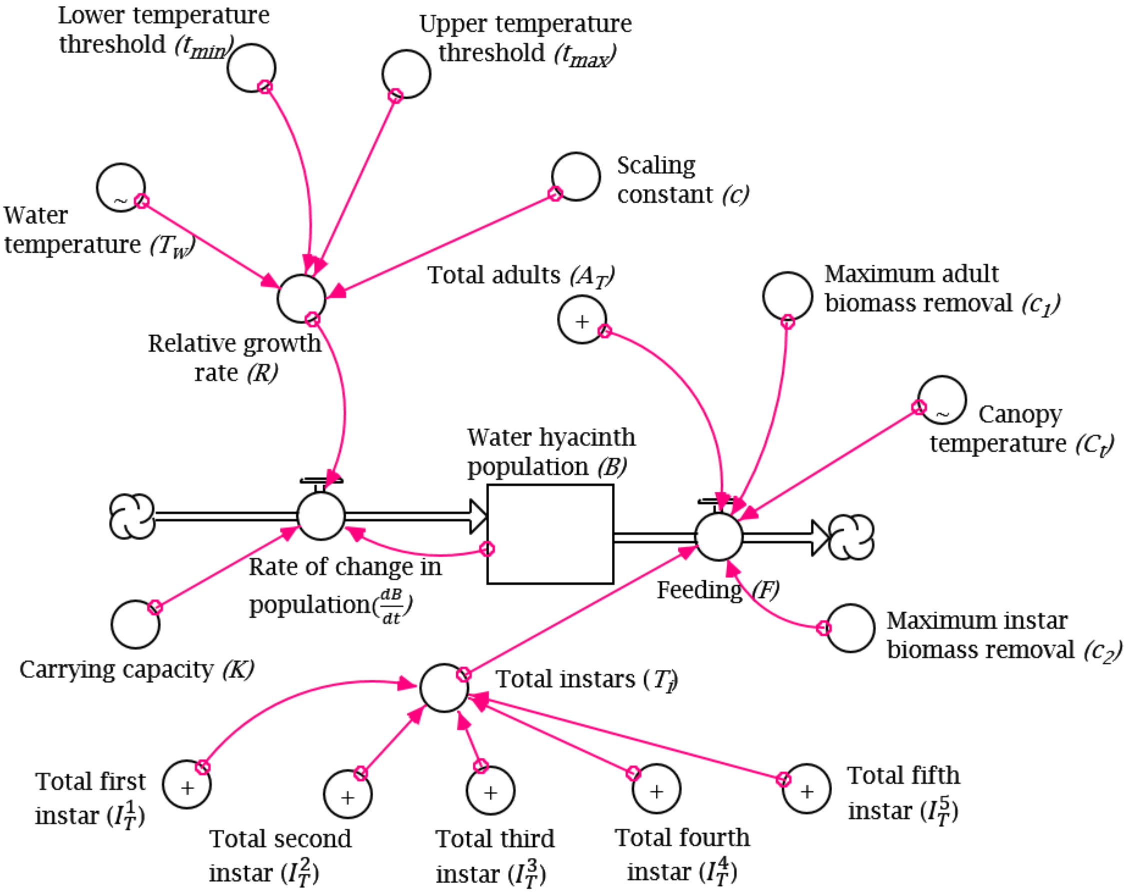


Figure 6.9. Logistic model of water hyacinth using a relative growth rate (R) based on water temperature (T_w) incorporating the feeding (F) using maximum biomass removed by *C. aquaticum* adults (c_1) and instars (c_2). The adult biomass removed (c_1) incorporated daily canopy temperature (C_t) while instar maximum biomass removed was a constant variable, based on biomass removal in the native range (Franceschini et al., 2011b). Layered conveyor, inflows and converters denote variables have been arrayed.

Parameter testing

The models described thus far have formed the base models for water hyacinth and *C. aquaticum*. These base models were then manipulated to better understand how changes to different parameters would impact the overall biomass density and number of adults at each of the field sites, after one year. A range of intrinsic growth rates (0.03, 0.04, 0.05, 0.06, 0.07, 0.08 g/g/day) and different starting densities (5000, 11250, 17500, 23750, 30000 g/m^2) were used for water hyacinth to represent the range of growth rates reported in the literature that could potentially occur in South African water bodies (Madsen, 1993; Heard and Winteron, 2000; Methy et al., 1990; Sale et al., 1985; Kobayashi et al., 2008; Eid and Shaltout, 2017). Simulations of these models of water hyacinth grown under different estimates of intrinsic growth (r_B) and different starting biomasses were performed to identify the range of responses that may be occurring at different field sites with different temperatures or other abiotic factors (Appendix 3, Appendix 4). Multiple population persistence models of *C. aquaticum* using different oviposition rates (0.5, 0.75, 1, 1.25, 1.5 eggs/female/day) and as well different survival proportions (0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) at the egg and various instars were performed. This was performed by running the base model while changing only one parameter at a time. For example, if the survival proportion of eggs was set to 0.5, then temperature dependent parameters, such as the oviposition rate and survival proportion of the different instar stages, were kept according to their original description. The oviposition rates chosen were to show the range of likely oviposition rates that *C. aquaticum* would be capable of, given the number of egg packets and eggs per packet shown in Chapter 5 and in the literature (Hill and Oberholzer, 2000;

Oberholzer and Hill, 2001; Vieira and Santos, 2003; Bownes, 2009). For example, the oviposition rate of 1.5 eggs/female/day relates to 547 eggs per year which is at the high-end of the range observed under warm quarantine conditions. These models were compared using the final number of adults at each site after one year.

Results

Water hyacinth growth

The daily average (mean ± SD) water temperature at the Golden Citrus site was significantly warmer than at the Farm Dam site, 21.99 ± 4.40 °C and 14.78 ± 5.05 °C respectively ($t_{2,703}$ =20.55; $p < 0.0001$). In the intrinsic growth model, the carrying capacity of water hyacinth was reached within 166 days and the rate of population change reached a maximum of 958 g/g/day, before decreasing to zero as the carrying capacity limited growth (Figure 6.10).

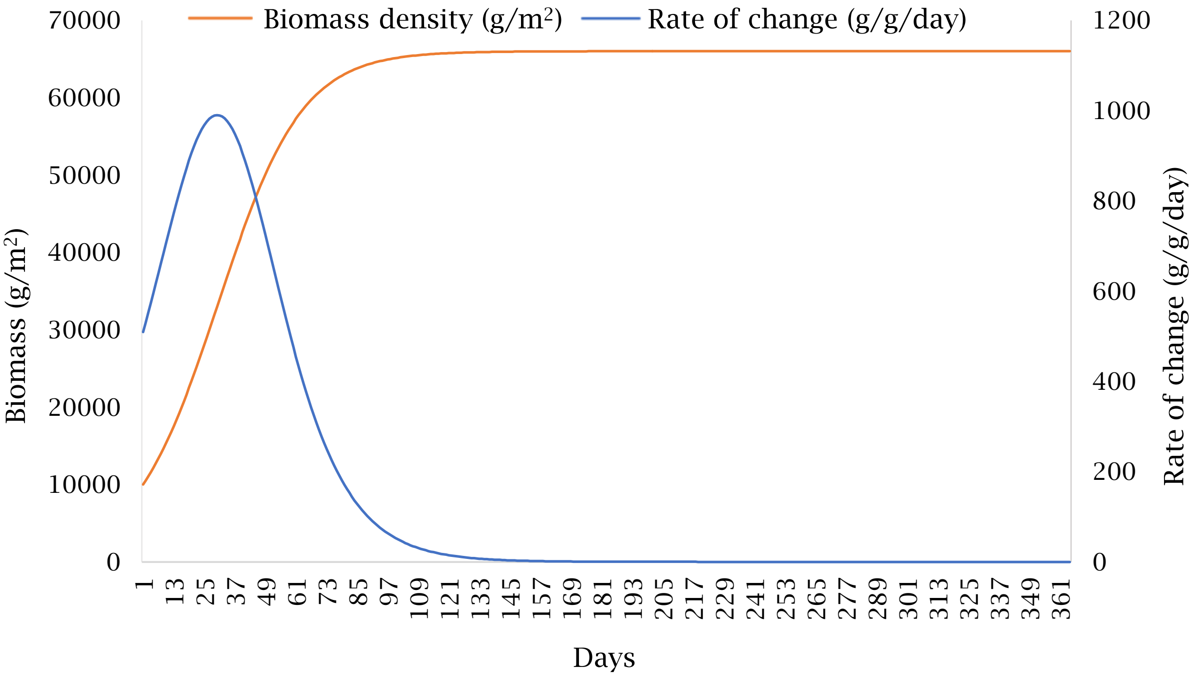


Figure 6.10. Logistic growth model of water hyacinth. Biomass density (g/m^2) is represented by the orange line and rate of change (g/g/day) of water hyacinth is represented by the blue line. The starting biomass density and intrinsic growth rate are 1000 g/m^2 and 0.06 respectively.

The water hyacinth biomass in the temperature dependant growth model reached carrying capacity in 158 and 306 days for Golden Citrus and Farm Dam respectively (Figure 6.11). At both sites the relative growth rate of water hyacinth dropped during winter, however only Farm Dam had temperatures that resulted in a growth rate of zero. The range of the relative growth rates based on water temperature of South African water bodies appears to range from 0.035 g/g/day and 0.06 g/g/day for most of the year between colder and warmer field sites.

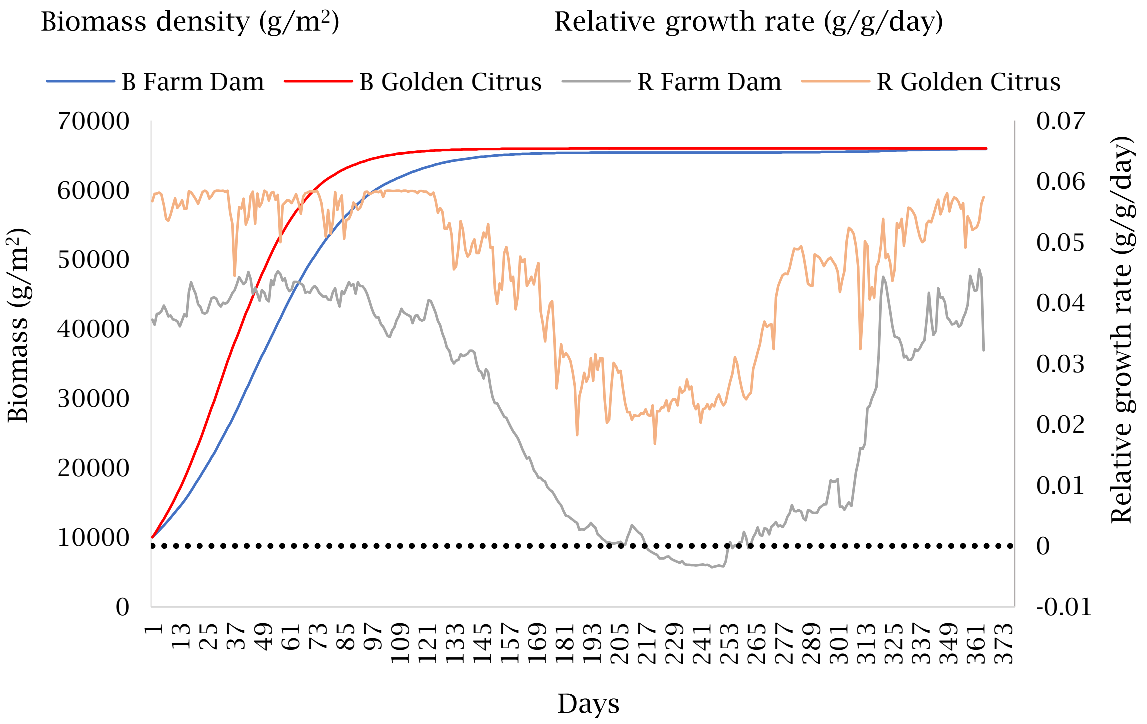


Figure 6.11. Logistic temperature dependent growth model of water hyacinth using water temperature data from Golden Citrus and Farm Dam. B and R represent the biomass density (g/m^2) and relative growth rate (g/g/day) of water hyacinth. Starting biomass density and carrying capacity were set to 10000 g/m^2 and 66000 g/m^2 respectively. The dotted black line denotes when the growth rate is 0.

Oviposition rate and egg survival

The daily average (mean ± SD) canopy temperatures at the Golden Citrus and Farm Dam sites were 21.43 ± 4.54 °C and 14.93 ± 5.44 °C, which was found to be statistically different ($t_{2,703} = 17.4$; $p < 0.0001$), thus the sites may be compared as one is considered a warmer water hyacinth site (Golden Citrus) and the other a colder water hyacinth site (Farm Dam). The simulated average (mean ± SD) oviposition rates by *C. aquaticum* adults was 1.28 ± 0.86 eggs/female/day at Golden Citrus and 0.46 ± 0.45 eggs/female/day at Farm Dam (Figure 6.12). The model predicts that for large portions of the year, oviposition rate is suppressed heavily by low temperatures, particularly at Farm Dam.

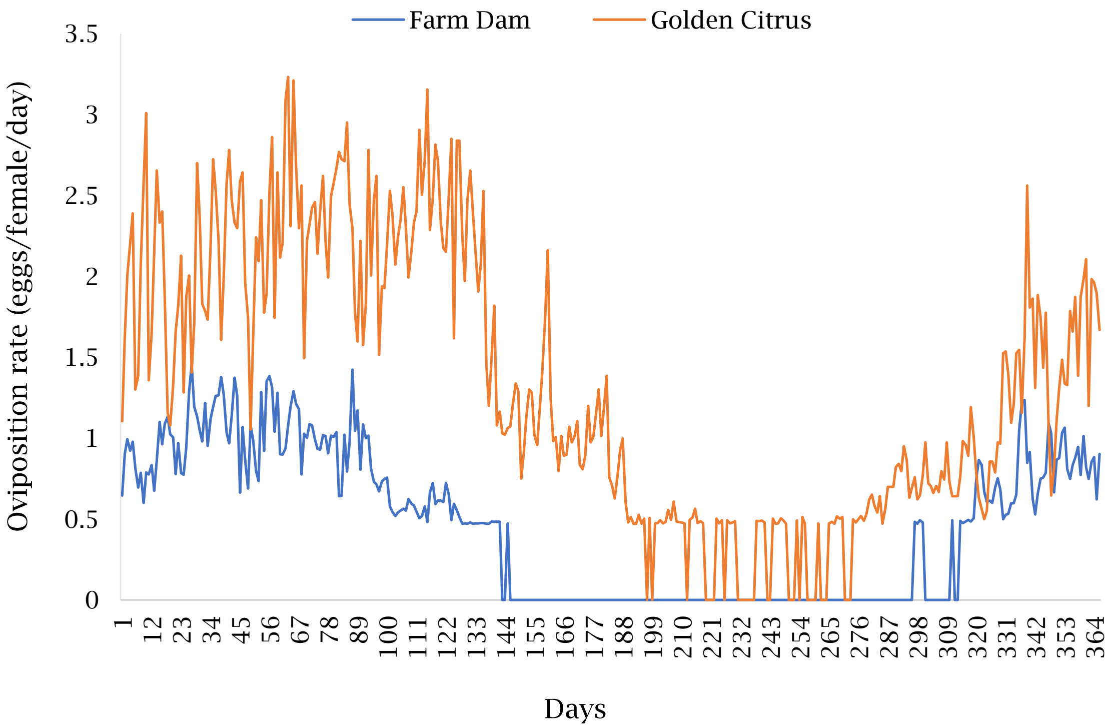


Figure 6.12. Simulated *C. aquaticum* oviposition rates based on field site canopy temperatures for Golden Citrus and Farm Dam after one year. Day one starts on December 1.

Average simulated (mean ± SD) egg survival proportions were low at both Golden Citrus and Farm Dam at 0.37 ± 0.14 and 0.23 ± 0.13 respectively (Figure 6.13). As with the oviposition rates, egg survival proportions will be heavily diminished by temperature for a large part of the year.

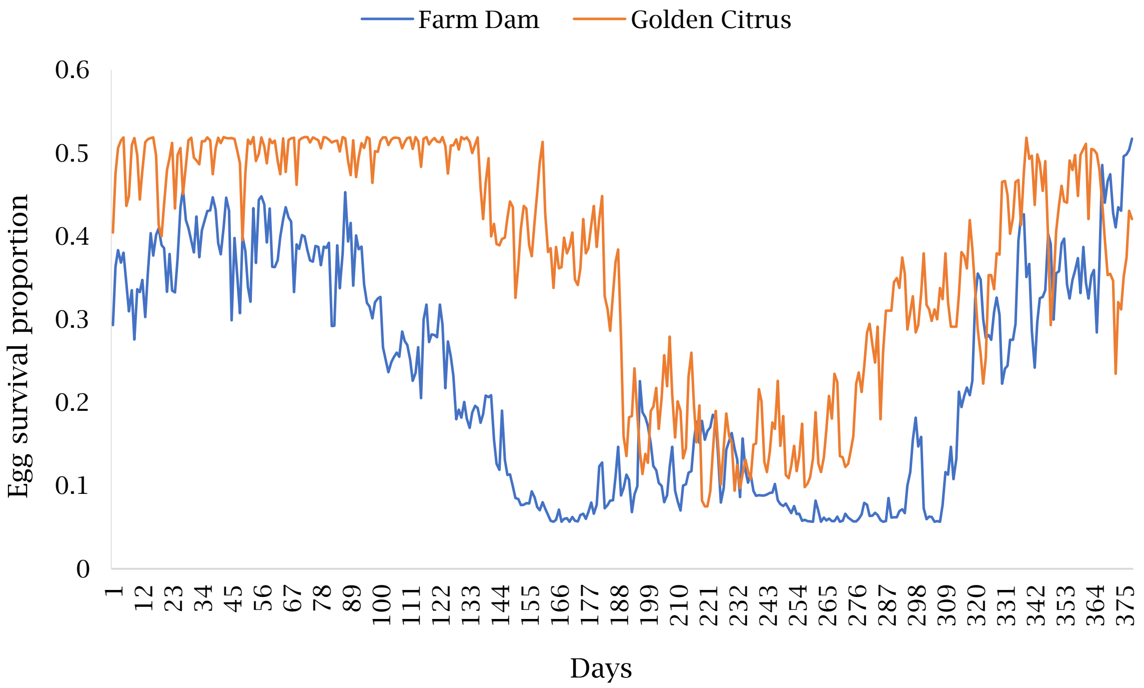


Figure 6.13. Simulated *C. aquaticum* egg survival based on field site canopy temperatures for Golden Citrus and Farm Dam after one year. Day one starts on December 1.

Cornops aquaticum development and carrying capacity

Simulated *C. aquaticum* adult and instar populations at Farm Dam completed their development from egg to adult in 95 days, at a daily average canopy temperature of 14.93 ± 5.44 ºC. However, the adult population after one year was smaller than the number of adults initially released (Figure 6.14). At Golden Citrus, development from egg to adult occurred in 73 days, at a daily average canopy temperature of 21.43 ± 4.54 ºC. The hypothetical *C. aquaticum* adult and instar population at Golden Citrus grew rapidly, reaching their respective carrying capacities in 157 days and 91 days respectively (Figure 6.15).

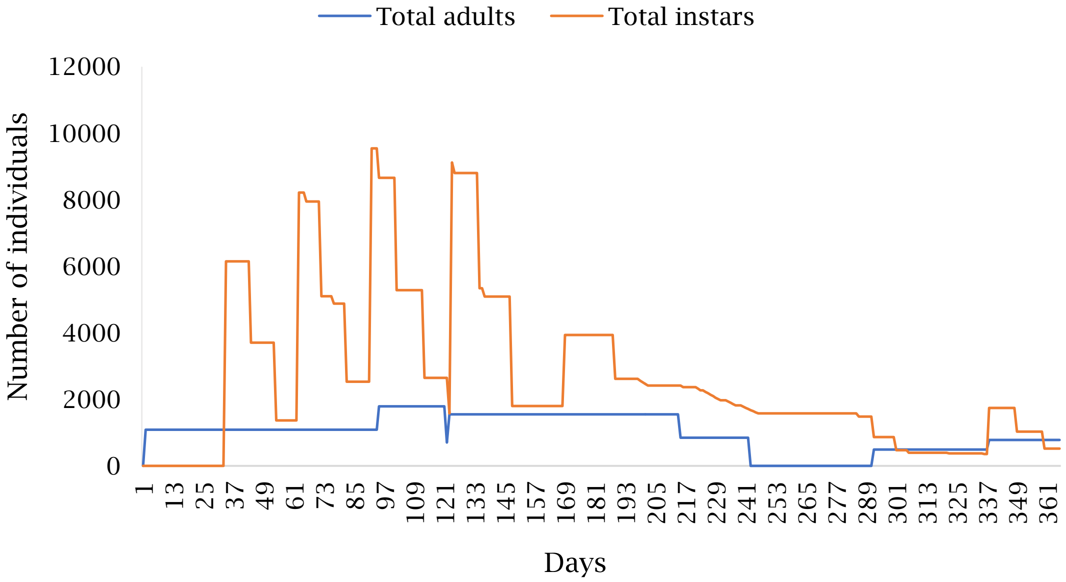


Figure 6.14. Simulated population numbers of *C. aquaticum* adults and instars at the Farm Dam site after one year, utilizing temperature dependent survival and oviposition. Day one starts on December 1.

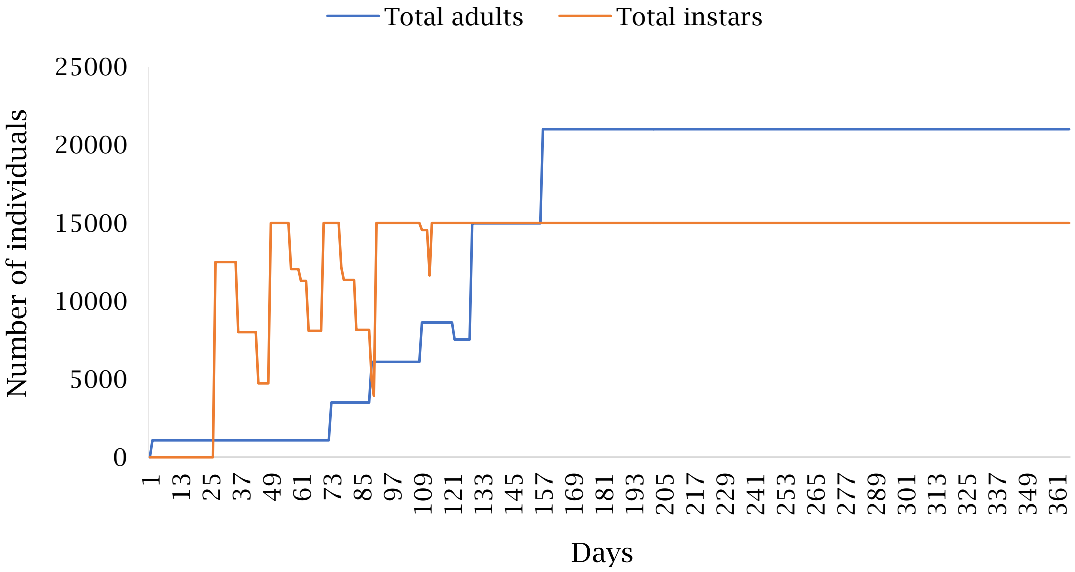


Figure 6.15. Simulated population numbers of *C. aquaticum* adults and instars at the Golden Citrus site after one year, utilizing temperature dependent survival and oviposition. Day one starts on December 1.

Manipulations of the oviposition rates of *C. aquaticum* showed that a minimum of 1.5 eggs/female/day would be required to maintain or increase the number of adults at Farm Dam over one year (Figure 6.16). Simulations for the Golden Citrus site showed 0.5 eggs/female/day would result in an increase in the number of adults present at the site after one year.

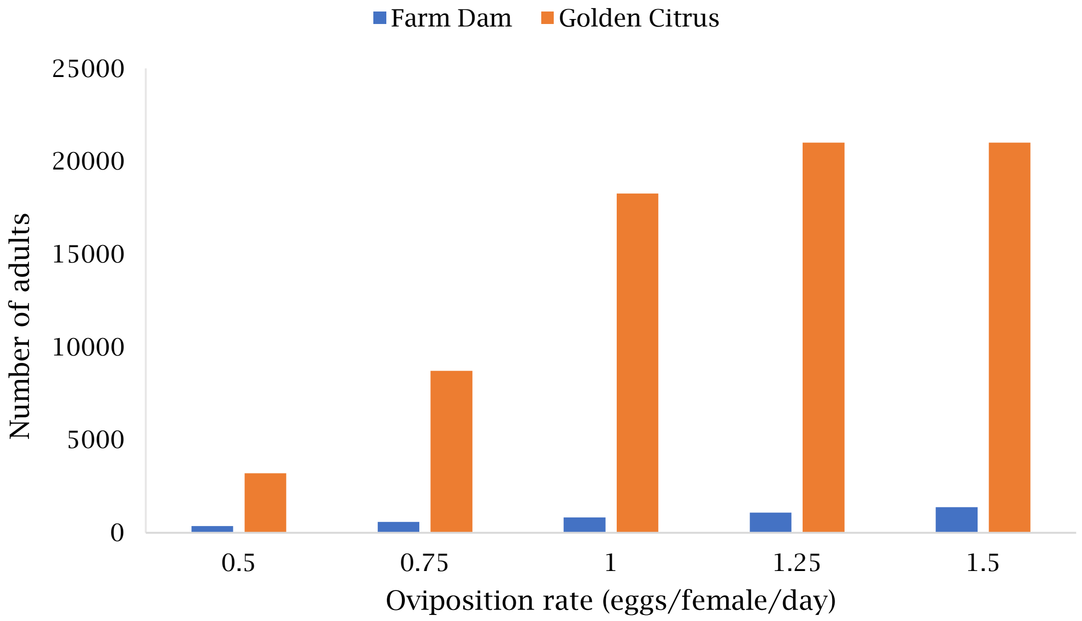


Figure 6.16. Simulated adult numbers resulting from different hypothetical oviposition rates at Golden Citrus and Farm Dam after one year.

Manipulations of survival proportions of *C. aquaticum* egg and instar life stages highlighted that increasing survival proportions of eggs and the second instar stage are key to population persistence of *C. aquaticum* adults at Farm Dam (Figure 6.17). Constant survival proportions of 0.5 and higher for the egg, and second instar stage or 0.7 and higher for first instars would have resulted in the hypothetical establishment of *C. aquaticum* at Farm Dam, where number of adults after one year were 1000 adults or greater. The manipulation of survival proportions for Golden Citrus showed hypothetical establishment would occur at survival proportions of 0.3 or higher in the egg or any of the instar stages (Figure 6.18). A survival proportion of 0.6 or higher in the egg or any of the instar stages results in the number of adults reaching their carrying capacity after one year.

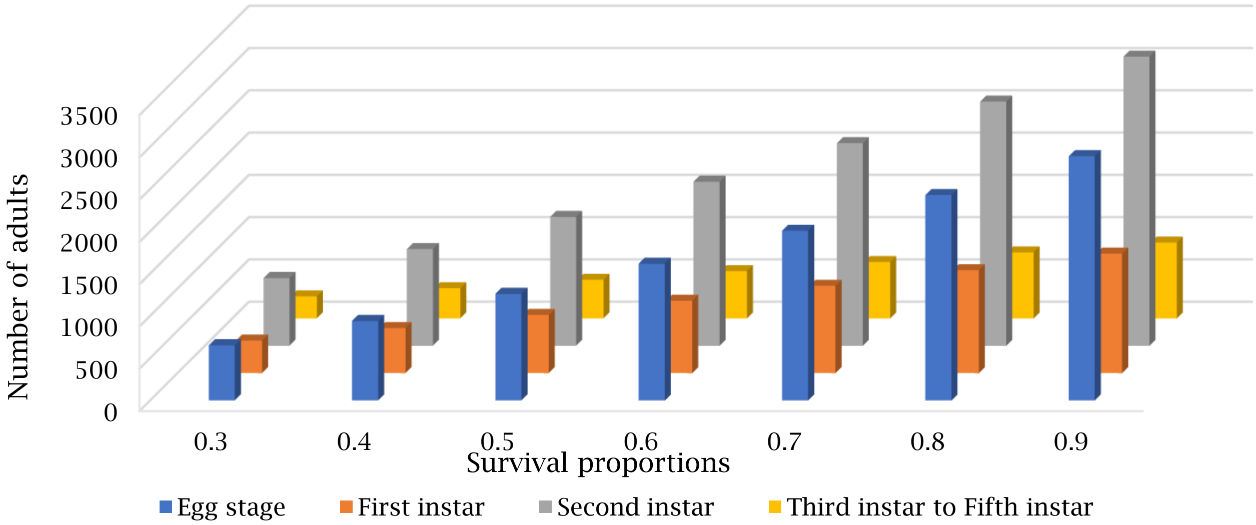


Figure 6.17. Simulated adult numbers with different hypothetical survival proportions (the proportion of individuals that survive that life stage) of egg stage, first instar, second instar and third to fifth instar stages at Farm Dam after one year. Each bar represents the model being run over a year with a single survival parameter being modified.

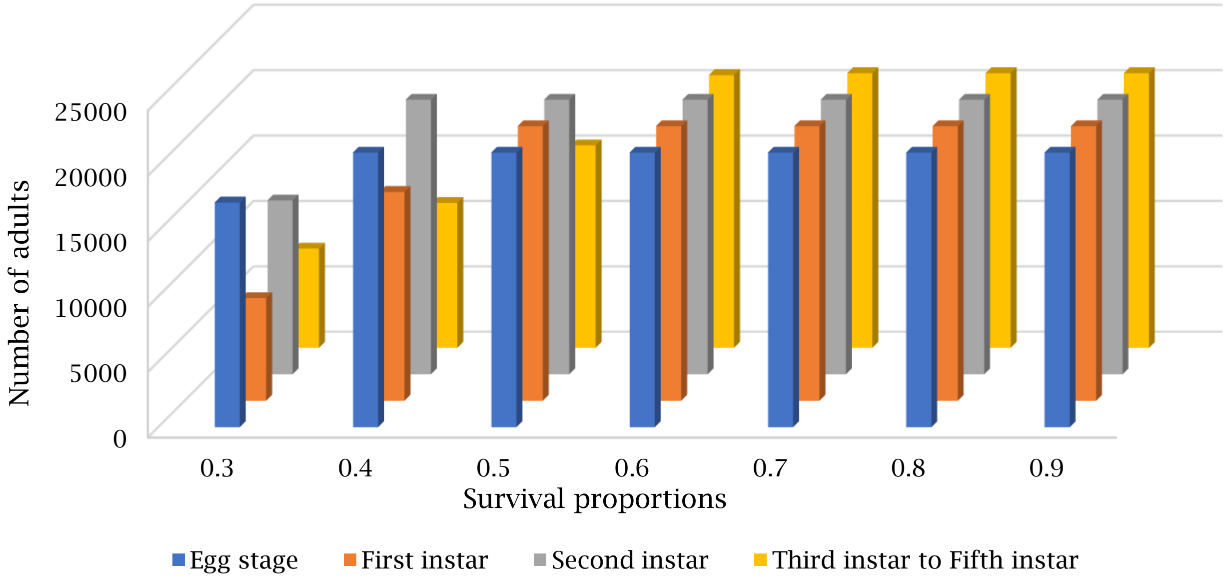


Figure 6.18. Simulated adult numbers with different hypothetical survival proportions (the proportion of individuals that survive that life stage) of egg stage, first instar, second instar and third to fifth instar stages at Golden Citrus after one year. Each bar represents the model being run over a year with a single survival parameter being modified.

Biomass removal by Cornops aquaticum

The hypothetical biomass removal by *C. aquaticum* adults on water hyacinth varied dramatically. At Farm Dam, when adult numbers were at their highest levels, a 10 % drop in water hyacinth biomass was noted (Figure 6.19). Biomass removal at Golden Citrus resulted in the complete removal of water hyacinth biomass over the year (Figure 6.20).

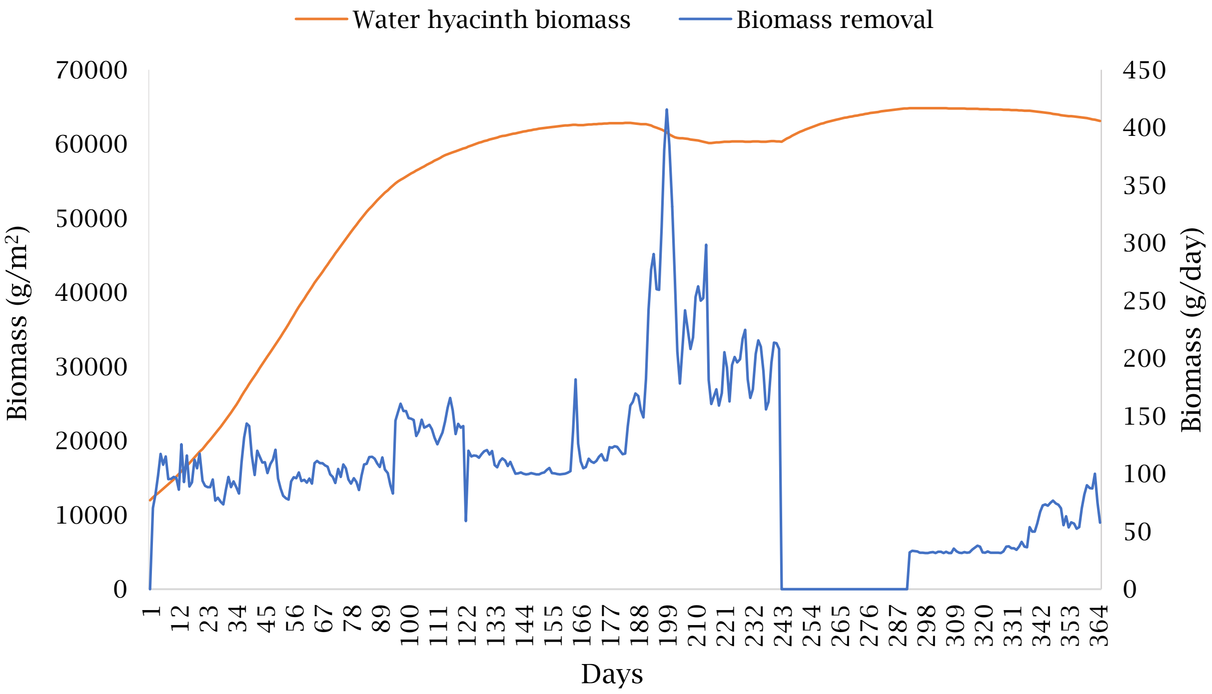


Figure 6.19. Simulated biomass removal by *C. aquaticum* on water hyacinth after one year, using daily canopy temperature driven maximal feeding damage at Farm Dam. Day one starts on December 1.

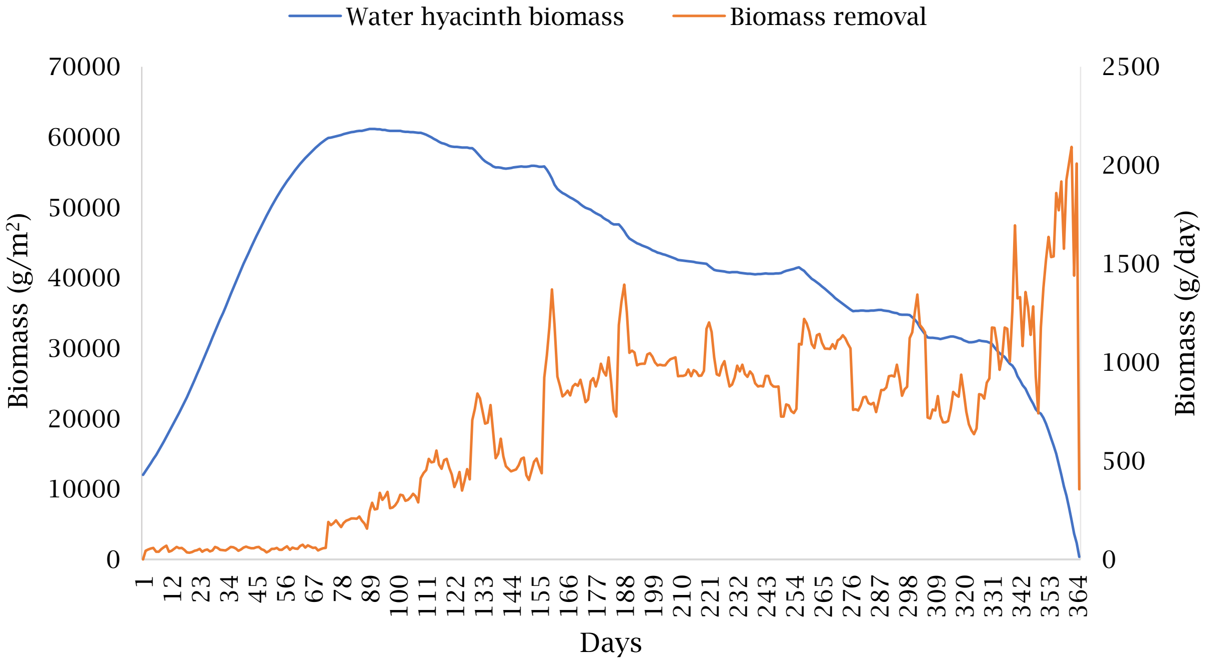


Figure 6.20. Simulated biomass removal by *C. aquaticum* on water hyacinth after one year, using canopy temperature driven maximal feeding damage at Golden Citrus. Day one starts on December 1.

Discussion

The models of water hyacinth biomass using an intrinsic growth rate and a relative growth rate based on temperature data showed an overall similar pattern, with the carrying capacity being reached in less than 130 days at a low starting density of 10000 g/m^2. This is consistent with other invasive aquatic plants and other models of water hyacinth (Wilson et al., 2005; Jacobs and MacIsaac, 2009; Hauptfleisch, 2016). The two sites chosen represented a wide range of water hyacinth field sites, highlighting some of the warmest and coldest water hyacinth sites in South Africa.

Given the logistical nature of the model, factors such as the water hyacinth biomass at a given time, the carrying capacity of water hyacinth and starting biomass might be significant considerations for control efforts given the state of water hyacinth biomass at different field sites (Tsoularis and Wallace, 2002; You et al., 2014). Once water hyacinth biomass reaches 50 % of its carrying capacity, the rate of change in the population reaches its maximum and with this, its' ability to compensate for loss of biomass by biological control agents (Tsoularis and Wallace, 2002; Soti and Volin, 2010). This highlights the importance of early detection of water hyacinth, monitoring protocols and attempts to maintain the population at the lowest possible level (Tipping et al., 2017; Jones et al., 2018). To improve the accuracy of the water hyacinth model, the addition of other factors such as the effects of dissolved nutrients and aspects of water hydrology such as flow velocity, water turbidity and sediment concentration should be added (Neiff et al., 2008; Köhler et al., 2010; You et al., 2014; Ochs et al., 2018).

The population increase of adult *C. aquaticum* at Golden Citrus and decrease at Farm Dam highlights the pronounced impact temperature can have on overall populations of the grasshopper (Kim et al., 2017). The difference noted in the population persistence at the two sites is likely the result of the large differences in egg survival and oviposition rates of *C. aquaticum* at the different sites. The high numbers of *C. aquaticum* adults attained in the simulation at Golden Citrus compared to the lack of establishment (Anthony King, pers. comm.) in the field stresses that the model does not accurately represent field populations. Despite this, the model can still improve understanding on the current system with regards to the survival and fecundity and guide further studies.

Fecundity could play a key role in *C. aquaticum* establishment given the variation in simulated oviposition rates at the different sites and the population persistence at Farm Dam under a higher fecundity model. The oviposition rate required to have the population persistent at colder sites, such as Farm Dam, was 1.5 eggs/female/day, assuming survival proportions remain the same. However, based on the results of Chapter 5, an average canopy temperature of 23.2 °C would be required for *C. aquaticum* females to produce 1.5 eggs/day compared to the actual canopy temperature of 14.9 ± 5.4 °C at the Farm Dam site. The fecundity estimates used in this model are based on the initial numbers of adults released and a set oviposition rate. Thus, a possible solution to increase the eggs entering the system even at low oviposition rates would be to increase the number of breeding adults through a larger initial release. However, given the high number of simulated adults released in the model, it may be advisable to rather make multiple initial releases (Grevstad et al., 2011).

The noted sensitivity of the third, fourth and fifth instar to changes in the survival proportion is likely an artefact of the model assumptions where survival was assumed to be high in these stages, similar to other grasshopper species. This suggests that the survival proportion of the third, fourth and fifth instar are likely much lower than presented in the model. In addition, the strong effect of temperature on the survival of eggs as well as the first and second instars likely extends to the later instars. Suitable weather conditions such as warm temperatures have been shown to be important in the survival of grasshopper instars, so much so that it has been considered a key factor in grasshopper outbreaks (Pickford, 1972; Branson, 2014).

The modelled elimination of water hyacinth at the Golden Citrus site after one year may be the result of the carrying capacity estimation of *C. aquaticum* based on its native range being too high. South African water bodies may also have higher levels of predation or pathogens that does not affect *C. aquaticum* in their native range (Stiling, 1993; Krams et al., 2013; Schulz et al., 2019). Additionally, the model does not consider water hyacinth as a feeding source and as a habitat for *C. aquaticum*. Thus, the carrying capacities of adults and instars in the model are constant even when water hyacinth biomass has been reduced substantially. The elimination of water hyacinth is likely a product of the model as eradication does not commonly occur in biological control, particularly in the control of IAPs, but rather strongly suppresses invasive populations in the long-term (Headrick and Goeden, 1996; Hulme, 2020).

Key aspects and possible areas for improvement

This model highlights that development of *C. aquaticum* at warmer sites and even colder sites is possible and the high mortality of grasshoppers throughout development, as a result of temperature, is likely the main hindrance in establishment. The model also shows the potential benefits that the establishment of *C. aquaticum* could have on control of water hyacinth.

Models can provide insight into systems but as they are simulations of reality, there will likely always be components that could be added to improve the current understanding of the system (Box, 1979). Many components can be added to a model to add to its complexity and increase its similarity to the real world. The expansions of a model that increase the complexity should always attempt to examine assumptions of the system in question or improve the understanding. Suggestions of expanding the model made here should be pursued, but only if they improve the understanding of the system beyond what has been done here.

A thermal stress model should to be added to this model as based on the reductions of *C. aquaticum* in the outdoor population experiments (Chapter 4), the mortality rate during winter is likely higher than the conservative estimates made in this model and occur even when temperatures do not reach critical thermal limits. Adding a carrying capacity estimate to the number of eggs and the different instars, particularly early instars, would likely improve the accuracy of *C. aquaticum* population abundance estimates. The model assumed more eggs could always be oviposited and hatch even if the system was inundated with eggs and first instars. The feeding component of the model could be investigated in greater detail, potentially looking beyond the system as simply insects removing biomass but as a response by the plants based on the plant quality, temperature and presence of other biological control agents.

The feedback of the condition of the plant to the grasshopper could also be added, particularly the nutrient status of water hyacinth, as this has been shown to affect feeding rates, development rates and overall fecundity of *C. aquaticum* (Center and Dray, 2010; Bownes et al., 2013a; Mukarugwiro et al., 2018). Investigations of the potential introduction of microbes either through direct feeding or through the frass of *C. aquaticum*, which have been shown in the water hyacinth weevil to reduce photosynthetic rates as much as biomass removal (Venter et al., 2013). The feeding impact of *C. aquaticum* on water hyacinth should be adjusted to allow for thermal variation in the damage inflicted by the instars, to account for seasonal variations in feeding rate. An interesting addition to this model would be the use of an interferential model to predict how the use of multiple species may interfere with one another, particularly at different population densities, and how these may influence the biological control of water hyacinth (Ludwig et al., 1978; Caughley and Lawton, 1981). Empirical studies of these interactions have shown the impact of herbivory on water hyacinth by different agents can impact plant growth parameters in different ways and may have additive effects on the control of water hyacinth depending on the combinations of biological control agents used (Marlin et al., 2013).

Chapter 7

General discussion

Introduction

Water hyacinth is the most damaging invasive aquatic plant in South Africa, as a result of its extensive distribution, economic costs and environmental damage (van Wyk and van Wilgen, 2002; Coetzee et al., 2011; Hill and Coetzee, 2017). The biological control program for water hyacinth in South Africa has been active since 1974 and has seen the introduction of nine biological control agents (Hill and Coetzee, 2017; Paterson et al., 2019). Although these agents have reduced the negative impact of water hyacinth, it is not currently under complete control. This lack of control is likely due to the eutrophication of South Africa's water bodies allowing prolific growth of water hyacinth negating the impact of the biological control agents released against it (Reddy et al., 1989; Ripley et al., 2006; Coetzee et al., 2007; Hill and Coetzee, 2017). Another possible reason for the general lack of control is the variable climatic conditions over water hyacinth's range in South Africa which makes certain agents climatically unsuitable to areas for introduction (Andow et al., 1997; Hill and Olckers, 2001; Day and Zalucki, 2009; Zachariades et al., 2009).

Cornops aquaticum (Orthoptera: Acrididae) was released in South Africa as a potentially more damaging biological control agent in 2011 after 16 years in quarantine. However, despite multiple release attempts, *C. aquaticum* was unable to establish at any of the sites it was released at (Anthony King, pers.

comm.). The lack of establishment was assumed to be related to temperature, as temperature is a major factor influencing many biocontrol agents and the population had been reared in warm quarantine conditions for close to 80 generations. This thesis aimed to explore various components of the thermal physiology and life history characteristics of *C. aquaticum* and its implications on its establishment as a biological control agent to water hyacinth in South Africa. This research attempted to improve the approach to investigating climatic incompatibility and possible ways in which establishment could be improved. The aim of this chapter is to synthesise the main finding of the thesis, make suggestions for future release protocols and highlight the limits of this work and possible avenues to pursue future work.

Summary and synthesis of the major findings

The high CT_{min} found in *C. aquaticum* compared to other biological controls agents of water hyacinth likely negatively effects its establishment potential in water hyacinth sites in South Africa (Table 7.1). As multiple sites across South Africa (except in the KwaZulu-Natal province) experience lowest minimum daily temperatures below the CT_{min} of *C. aquaticum* (Figure 2.3). The similarity of the high critical thermal maximum (CT_{max}) of *C. aquaticum* to the water hyacinth weevils and the mite *Orthogalumna terebrantis* (Acarina: Galumnidae) suggest warmer temperatures are unlikely to cause problems with establishment.

Table 7.1. Critical thermal minima (CT_{min}) of *Cornops aquaticum* and the other tested biological control agents of water hyacinth.

Species	Common name	CT_{min} (ºC, mean)	Reference
Cornops aquaticum	Water hyacinth grasshopper	6.29 ºC (males) 6.01 ºC (females)	Chapter 2
Eccritotarsus catarinensis	Water hyacinth mirid	1.2 ºC	Coetzee et al., 2007
Orthogalumna terebrantis	Water hyacinth mite	3.16 ºC	Marlin, 2011
Megamelus scutellaris	Water hyacinth plant hopper	4.7 ºC	Coetzee, unpublished
Neochetina eichhorniae	Water hyacinth weevil	1.1 ºC	Reddy et al., 2019

The lower CT_{min} in retested and acclimated groups highlights the ability of the species to undergo acclimation. Acclimated individuals had significantly faster recovery time from chill coma than individuals from the stock. Therefore, *Cornops aquaticum* can acclimate to colder conditions if winter temperatures progressively drop allowing time to acclimate. This would extend their thermal range and recovery time from chill coma, making them less vulnerable and more likely to survive at colder temperatures. However, the degree of acclimation possible and the trade-offs of these acclimations are unknown. One such problem is likely that numerous factors are impacting the thermal performance of *C. aquaticum* and acclimation may not solve them all sufficiently (Kingsolver and Woods, 2016). Such that, if *C. aquaticum* were to acclimate to temperatures colder than its critical thermal minima, their activity levels at cold temperatures may remain low (as a result of a reduced metabolic rate) and this is likely to increase predation risk and reduce overall fitness and fecundity. The longer durations at sub-lethal temperatures suggest that adult survival at warmer sites, where minimum temperatures were above 7 ºC, would be possible during colder winter conditions.

The mass-specific metabolic rate (VCO_2 µl.hr^{-1}.g^{-1}) of adult *Cornops aquaticum* was 1139.5 ± 89.97 at 25 ºC. The metabolic rate of *C. aquaticum* was higher than other closely related species of grasshopper (Chapter 3) and may be optimised for competition and predator avoidance. The metabolic rate of adults was significantly lower at 15 ºC than at warmer temperatures (25 ºC and 35 ºC). At 35 ºC the immature stages increased their mass-specific metabolic rate (974.76± 161.80 µl.hr^{-1}.g^{-1}) to almost five times that exhibited at 15 ºC (200.63 ± 60.20 µl.hr^{-1}.g^{-1}) and 25 ºC (292.04 ± 389.94 µl.hr^{-1}.g^{-1}). Body mass was correlated with metabolic rate for adults at 25 ºC, but not at 15 ºC and 35 ºC while the metabolic rate of immatures showed a strong relationship with body mass, which strengthened with rising temperatures (15 ºC, 25 ºC and 35 ºC). Both adult and immature stages of *C. aquaticum* showed a largely reduced metabolism at 15 ºC which reflects the warm climate in their native range and suggests the species is better suited to warmer temperatures. This suggests grasshoppers will likely have largely reduced activity levels during winter, even during mild winter conditions which may result in higher mortality (Guppy, 2004; Laloutte et al., 2011; Kelly et al., 2014).

Despite longer durations at sub-lethal temperatures suggesting survival through winter at temperatures greater than the CT_{min} was possible, the outdoor population mortality (Chapter 4) and the mass loss of adults in the feeding experiment at 15 ºC (Chapter 5) suggests otherwise. Increased thermal stress through longer durations at sub-lethal temperature likely contributes to winter mortality. This probably occurs through the accumulation of thermal stress or through a gradual loss of condition, increasing the likelihood of mortality by other means (Kingsolver and Woods, 2016; Lubawy et al., 2019).

The temperature limits and metabolic investigation provides a good basic test for establishment potential, possibly eliminating some sites from release attempts. However, these thermal physiology components do not account for the behavioural responses of *C. aquaticum* and the temperature experienced by the grasshopper at a certain ambient temperature. For example, adult *C. aquaticum* appear to make use of thermal refuges through microhabitat selection on and around the water hyacinth plants, and immature *C. aquaticum* increase their body temperature through basking on the upper leaves of water hyacinth. The testing of the body temperature compared to the substrate temperature and the position on the plant at different ambient conditions, allows a greater understanding as to how the grasshopper tolerates the environment it is in. Whilst significant losses by the caged outdoor population were noted (> 60 % in adults and instars from May to July in 2017 and in 2018), the population was able to persist, over nearly two winter seasons (15 months).

The mortality of the caged *C. aquaticum* population occurred despite mean canopy temperatures throughout the winter being still within the behavioural range of *C. aquaticum*. The mortality is likely a product of accumulated thermal stress due to the insects experiencing periods at sub-lethal temperatures. Adult *C. aquaticum* were able to maintain their body temperature significantly higher than substrate temperatures in spring and summer, but not in winter. This highlights their likely inability to maintain their body temperatures well above the winter substrate, which may cause individuals to move off the plant, seeking a thermal refuge or experience higher mortality (Franceschini et al., 2011a; Kingsolver and Woods, 2016; Lubawy et al., 2019). The immatures did not maintain their body temperature significantly higher than the substrate temperatures during both spring and winter. The inability of immatures to maintain body temperatures significantly higher than the substrate temperature, is likely a result of their smaller body size compared to adults.

Due to the importance of temperature on the metabolism, and winter survival and persistence of *C. aquaticum*, investigations on the effect of temperature on life history characteristics including feeding rates, development, oviposition rates and survival of *C. aquaticum* were performed (Chapter 5). Water hyacinth removal rates by *C. aquaticum* adults was 0.09 ± 0.036 g/grasshopper/day and were similar to the measurements of *C. aquaticum* in its native range (Capello et al., 2011; Franceschini et al., 2011b). Feeding experiments on whole plants highlighted that only at 25 °C did *C. aquaticum* have a significant negative impact on the growth of water hyacinth. Despite removal rates on individually cut leaves showing increased removal rates at 30 °C and 35 °C, whole plants of water hyacinth were able to compensate for damage by *C. aquaticum* at 35 °C. These results indicate the importance of temperature as a mediating factor in the feeding rates of *C. aquaticum* and highlighted the importance of understanding the possible compensation in plant-insect interactions. The body mass of *C. aquaticum* increased at 35 °C compared to 25 °C and 15 °C which may indicate improved fecundity of *C. aquaticum* at higher temperatures. This may be particularly important for females, as body mass has been linked with increased survival and fecundity (Adis et al., 2008; Garrad et al., 2016; Walczak et al., 2017). Whilst the loss of body mass and low feeding rates at 15 °C clearly corresponds with the disproportionately low metabolic rates measured at 15 °C (Chapter 3) and may explain the significant mortality in the outdoor population during winter (Chapter 4).

The optimal temperature for egg development and survival was found to be 25 ºC and 30 ºC, with much lower egg survival and slower development noted at temperatures below 20 ºC and above 35 ºC. The narrow optimal egg development temperatures correspond to the ambient canopy temperatures at most of the water hyacinth field sites in South Africa during the spring and summer (Byrne et al., 2010). The overall number of egg packets (4.0 ± 0.9) and the eggs within the egg packets (29.6 ± 14.2) is similar to those found by Bownes (2009) but lower than those noted by Hill and Oberholzer (2000) for South African *C. aquaticum*. In the native range of *C. aquaticum*, Vieira and Santos (2003) reported only 13 ± 5 eggs per egg packet. This variation in fecundity may highlight the effect of plant choice and quality and intra-populational differences between individuals (Ferreira and Vasconcellos-Neto, 2001; Capello et al., 2011). At 25 ºC the developmental duration of *C. aquaticum* instars was similar to the duration in their native range, however the fastest developmental rates were observed at 35 ºC (Vieira and Santos, 2003; Capello et al., 2011; Capello and Franceschini, 2014). High mortality was observed even at more favourable temperatures, with 58 % survival of eggs and 40 % survival of instars to adulthood at 25 ºC. Both plant quality and photoperiod having been shown to influence mortality (Adis et al., 2004; Zerbino et al., 2013; Nissinen et al., 2017). The photoperiod (12:12 L:D) used in this study, although a good average day length, may have led to lower survival to adulthood measures than other studies (Adis et al., 2004; Bownes, 2009; Zerbino et al., 2013). The constant temperatures may also have negatively affected development, as fluctuating temperatures compared to constant temperatures have been shown in some species to result in faster development (Worner, 1992; Milosavljević et al., 2019).

Simulations of water hyacinth growth and *C. aquaticum* establishment at water hyacinth field sites, with the inclusion of *C. aquaticum* development, fecundity and survival data (Chapter 5), were modelled in Chapter 6. Mathematical models of the population dynamics of water hyacinth and *C. aquaticum* may improve the understanding of the system as a whole, and allow for improved approaches for the release and establishment of the agent. By comparing two water hyacinth field sites, of different average and minimum temperatures, both of which experience winter minimum temperatures that reach or exceed the critical thermal minima of *C. aquaticum*, the effect of temperature on life history characteristics were explored.

Without any biological control, the growth of water hyacinth at both sites increases quickly until it reaches its carrying capacity, 158 and 306 days for Golden Citrus and Farm Dam respectively. Simulated oviposition and egg survival proportions of *C. aquaticum* using field site temperature data reveals how winter temperatures, particularly at Farm Dam, suppress potential population growth. Although *C. aquaticum* could complete development at Farm Dam, the population number after one year was less than the original release number. Simulations at Golden Citrus of *C. aquaticum* showed quicker development than at Farm Dam, and reached their carrying capacity within half a year.

The simulated biomass removal of *C. aquaticum* was extensive, and showed that at both sites, the impact of feeding by *C. aquaticum* was able to reduce the water hyacinth population to below its carrying capacity. The fact that simulated *C. aquaticum* reached high population numbers at Golden Citrus despite no establishment occurring at the field site, suggests the model does not accurately represent real field populations. This is most likely due to the low survival proportions of instars which may be improved with changes to the release protocol.

Suggestions for release protocol and establishment

Given the sensitivity of *C. aquaticum* to colder temperatures, changes in laboratory rearing temperatures or the use of acclimation trials before release attempts, may prove beneficial. Rearing at colder temperatures may select for individuals capable of handling the wide range of temperatures in the field, thus preventing adult mortality when there are sudden drops in temperature (Wright and Bennett, 2018). In addition, sites which have consistently higher spring and summer temperatures may assist the development of *C. aquaticum* eggs and instars. In the simulations of Chapter 6 the *C. aquaticum* adult and instar population growth at a warm water hyacinth site quickly reached their carrying capacity, highlighting the establishment potential at many water hyacinth field sites in South Africa. Water hyacinth sites that *C. aquaticum* is more likely to establish at, based on the lowest minimum daily temperature and the physiology of *C. aquaticum* are: Princess Vlei, New Years Dam, Golden Citrus, Mkadhzi Spruit and all sites in KwaZulu-Natal.

If individuals of the current quarantined population of *C. aquaticum* were to be released without any “renaturalising” it is suggested that all releases be focused on one or two field sites where temperatures are warmest. In addition, multiple large releases throughout spring and summer may assist in providing a large, consistent egg, instar and adult inflow, while allowing the population to undergo adaptation to the local conditions. The first attempts at *C. aquaticum* release in 2011 were at four different sites with 300 individuals released per site (Bownes et al., 2011). It is suggested a minimum of 1000 individuals (adults and late stage instars) are released augmentatively over a summer season, in order to inundate the field site with eggs and to accommodate for the high winter mortality that was observed in Chapter 4, despite temperatures not reaching the critical thermal minima of *C. aquaticum*. Releases should continue until the population has established and shows persistence through winter.

Studies with the water hyacinth mirid, *Eccritotarsus catarinensis* (Hemiptera: Miridae) have highlighted two key aspects which may pertain to *C. aquaticum*. Genetic distinctions were found between individuals from Peruvian populations compared to Brazilian populations of *E. catarinensis* (Taylor et al., 2011; Paterson et al., 2019). The genetic variation in naturalised populations (located at Nseleni River and Mbozambo Swamp, KwaZulu-Natal) of *E. catarinensis* released from the Plant Protection Research Institute (PPRI) in Pretoria, whose culture was founded from a single gravid female (Martin Hill, pers. comm.) were almost as high as those in the Peruvian population (Taylor et al., 2011). The difference between the Peruvian and Brazilian *E. catarinensis* populations suggests different *C. aquaticum* populations across South America may include a suite of genetically diverse *C. aquaticum* populations. Furthermore, the impact of 16 years of quarantine before release likely negatively affected the genetic variation of *C. aquaticum* resulting in difficulties establishing (Adis et al., 2008; Bownes et al., 2011). The addition of genetic variation, either through the recollection of native populations or the "renaturalisation" of the species, is likely necessary in order to improve the likelihood of establishment.

The preferred approach for release and establishment is a large scale "renaturalisation" of the quarantined population of *C. aquaticum* in an effort to increase the genetic diversity and reduce the negative effects of temperature-controlled laboratory conditions that inevitably occurred in the 16 years of quarantine, as well as increase the thermal tolerance of the population. This could be accomplished by simply having a warm field site that is monitored frequently and *C. aquaticum* adults are released regularly to maintain population numbers (similar to the caged outdoor population). Alternatively, an outdoor *C. aquaticum* enclosure could be set-up at one of the colder water hyacinths sites. Although mortality will be high initially, a portion of the population will acclimate and/or adapt to the cold environment. The offspring of these individuals should then be reared in quarantine at colder temperatures and used for release at other field sites.

Limitations and future work

Cornops aquaticum showed improved survival, oviposition, feeding damage and faster development with warmer temperatures, although this may fall within the variation exhibited by the species across its wide distribution. Therefore, all of the physiological measures tested should preferably be compared directly with individuals from the native population(s) of *C. aquaticum* in order to ascertain the effect of population differences between the native South American and South African populations. The physiological attributes measured in this thesis could also be measured during the selection of future biological control agents for water hyacinth, and possibly other aquatic IAPs. The high level of damage caused by the grasshopper to water hyacinth and the simulated ability to regulate water hyacinth populations in the model, show the potential control that could be added through further establishment efforts.

Future studies should investigate the reasons for the very high egg mortality and devise a method of increasing egg survival, as this is a key component to the lack of establishment of *C. aquaticum*. Further research of physiological parameters such as metabolic rates, thermal stress accumulation and critical thermal limits should be added to the model to better understand grasshopper survival during winter. The results of Chapter 4 have touched on the effect predators, such as spiders and birds, may have on the survival and persistence of *C. aquaticum* and other biological control agents. The role of predation on the survival and efficacy of water hyacinth's biological controls agents should also receive further investigation.

Winter temperatures in the southern extent of the distribution of *C. aquaticum*, such as Uruguay or Argentina, are cooler than other areas of South America. Establishment attempts using *C. aquaticum* individuals collected from these areas should be seriously considered as the colder conditions experienced may favour the more temperate regions of South Africa. Such a collection from Argentina was intended for the austral summer of 2008/2009 (Bownes, 2009) which unfortunately, did not occur. The collection of a cold tolerant strain of *C. aquaticum* is likely to benefit control efforts at both the warmer and colder sites of water hyacinth, as it may allow for rapid population growth of *C. aquaticum* at warmer sites thus compensating for water hyacinth's rapid growth at warmer temperatures.

The assumption that a wide native distribution is associated with a wide physiological tolerance still holds true theoretically, especially with species that show high phenotypic plasticity. However, if an organism, such as *C. aquaticum,* employs behavioural means in their native range to survive colder temperatures during winter, such as moving onto a thermal refuge (*Oxycaryum cubense*; Franceschini et al., 2011a), their ability tolerate colder temperatures in an introduced range may be reduced. However, this must be understood with the consideration that a wide native distribution may also suggest differences in the inter-populational physiological tolerances.

Conclusion

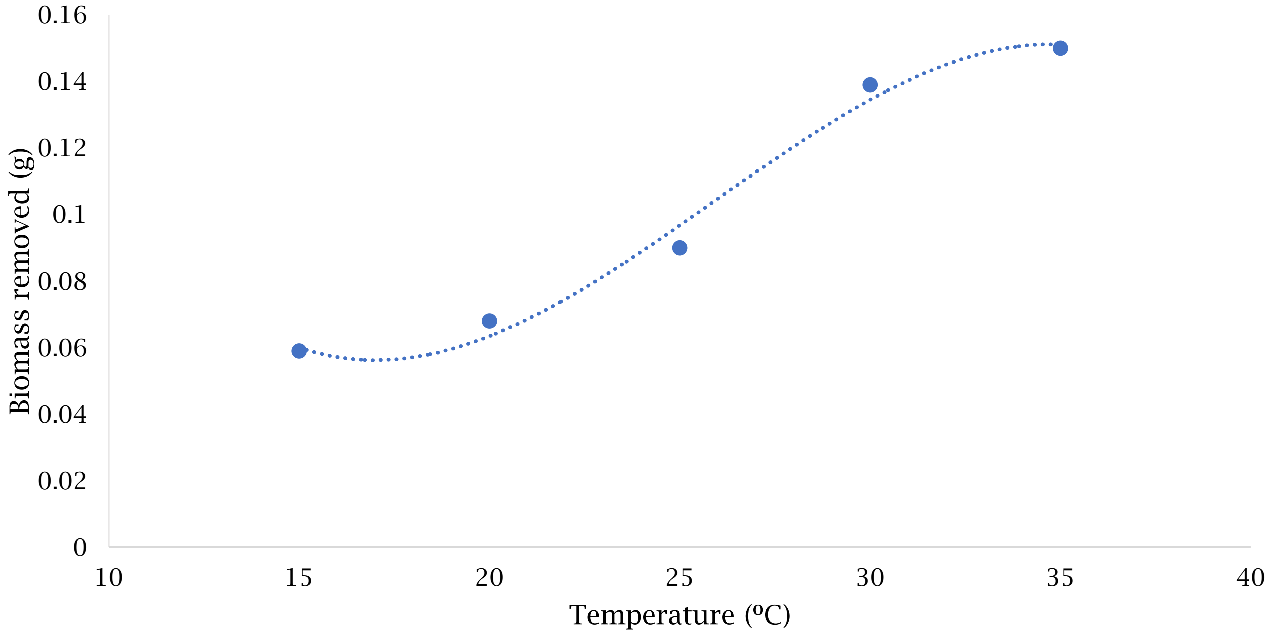
In conclusion, collections of cold tolerant *C. aquaticum* from southern South America should occur only if economically feasible. Establishment attempts with the current quarantined population of *C. aquaticum* should consider a "renaturalisation" of the population at a larger scale than attempted in this study (Chapter 4) and releases should focus on large release numbers at warm sites during spring and summer to saturate the field site with eggs which will counter the high egg mortality and high adult mortality during winter.

Appendix

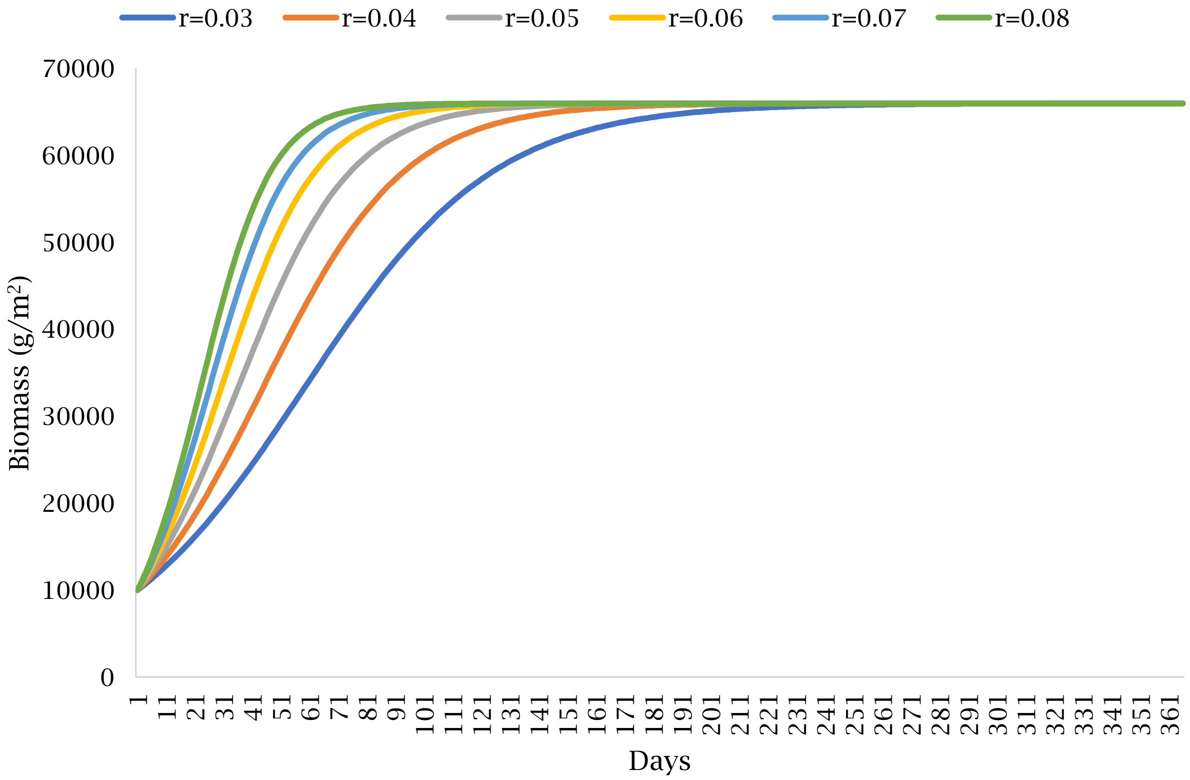
Appendix 1. Parameters for water hyacinth and *Cornops aquaticum* models

Parameter	Description	Units	Estimates
r_B	Intrinsic growth rate	g/g/day	0.06
B	Current biomass density	g/m^2	State variable
K	Water hyacinth carrying capacity	g/m^2	66000
R	Relative growth rate	g/g/day	Variable with temperature
c	Empirical scaling constant	-	8.7×10^{-6}
T_w	Water temperature	ºC	Variable
T_{min}	Lower temperature threshold	8 (ºC)	8
T_{max}	Upper temperature threshold	40 (ºC)	40
S_a	Specific day array	days	Variable
C_d	Day count	days	Variable
A_t	Adult number	individuals	State variable
A_i	Adults inflow	individuals	Variable with temperature
S	Senescence	days	120
M_5	Fifth instar moult	individuals	Variable with temperature
M_S^5	Instar 5 survival proportion	-	Variable
E_t	Egg	individuals	State variable
E_i	Egg inflow	individuals	Variable with oviposition
E_h	Egg hatching	individuals	Variable with temperature
E_t	Accumulated eggs	individuals	Variable with time

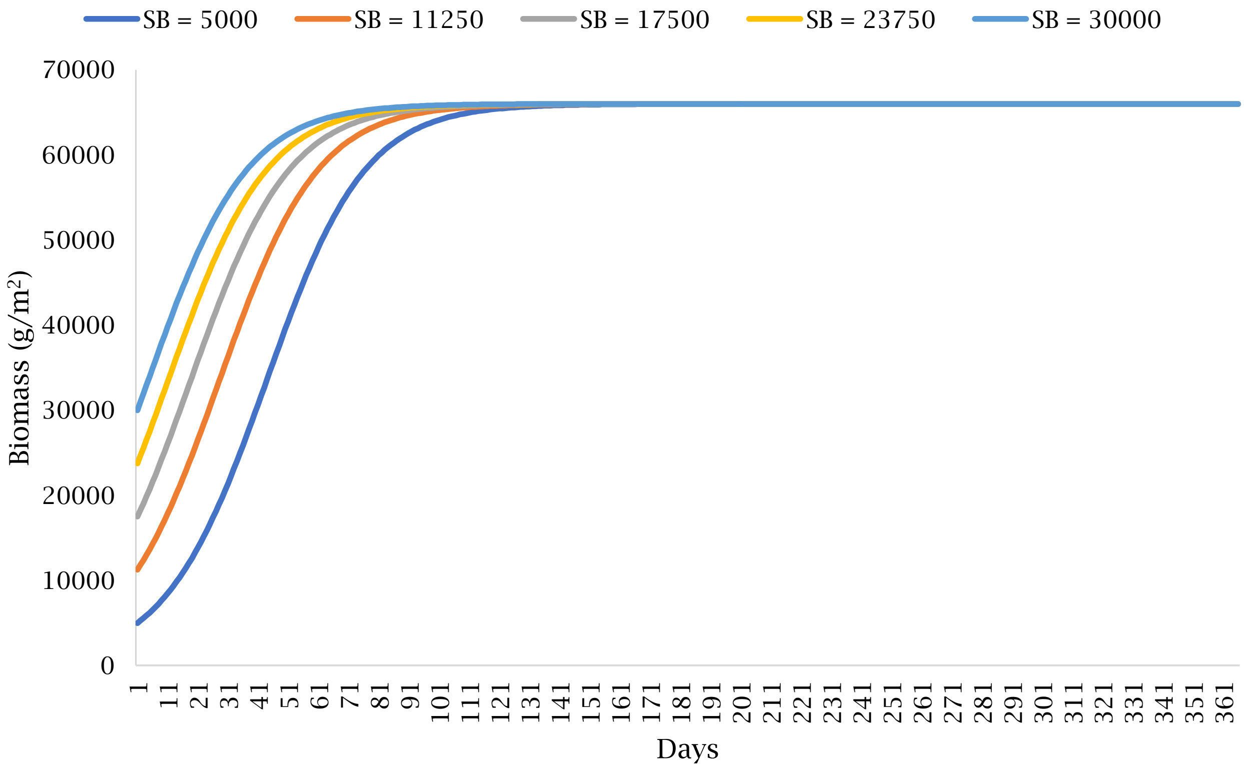
Parameter	Description	Units	Estimates
O	Oviposition rate	eggs/female/day	Variable with temperature
D_e	Thermal units	ºD	Variable
D_r	Degree day reset	ºD	Variable
C_t	Canopy temperature	ºC	Variable
t	Lower developmental threshold	ºC	Variable
CDD_{e_t}	Cumulative egg degree day	ºD	Variable
K_e	Egg thermal constant	ºD	396
E_s	Egg surival proportion	-	Variable with temperature
M_s^1	Instar survival proportion	-	Variable with temperature
$CDD_{i^1 t}$	Accumulated first instar degree day	ºD	Variable
I_t^1	Accumulated instars	individuals	State variable
I_i^1	Instar inflow	individuals	Variable with temperature
M_1	Moult	individuals	Variable with temperature
T_i	Total number of all instars	individuals	State variable
D_c	Critical thermal mortality	-	Variable with temperature
K_i	Instar carrying capacity	instar/m^2	5
K_a	Adult carrying capacity	adult/m^2	7



Appendix 2. Biomass removal of adult *Cornops aquaticum* at different temperatures. The dotted line represents a third order polynomial regression (y= -0.00003x3 + 0.0026x2 - 0.0602x + 0.4846: $R^2 = 0.9869$).



Appendix 3. Logistic growth model of water hyacinth biomass (g/m^2) under different intrinsic growth rates (r_v). Starting density was 10000 g/m^2



Appendix 4. Logistic growth model of Water hyacinth biomass (g/m^2) with different starting biomass densities. The intrinsic growth rate (r_v) was set at 0.06.

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