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The effect of temperature on biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae) in South Africa

Anthony Michael King



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

Johannesburg, 2011

DECLARATION

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



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Candidate – Anthony Michael King

18TH day of AUGUST 2011

ABSTRACT

The behaviour and physiology of every insect, during all developmental stages, is largely determined by temperature. Metabolic rate, flight activity, nutrition, growth rate, oviposition and longevity can all be correlated to temperature. Consequently, insect development occurs within a definite temperature range which can be experimentally determined. This serves as a basis from which models that estimate insect growth, development and reproduction can be formulated. Such studies on temperature-dependent development are therefore important for understanding predator-prey relationships and insect population dynamics relevant in epidemiology, pest management and biological control of weeds and insect pests.

The biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in South Africa currently relies on six established agents. However, the results of this programme do not compare well with the achievements made elsewhere. This has been attributed to a number of constraining factors, chief among which is a wide variety of climatic regions, low minimum temperatures and a high incidence of frosting which slows the build-up of natural enemy populations. This research verified and augmented the thermal tolerance data available for three of South Africa's more efficacious agents used against water hyacinth, namely *Neochetina eichhorniae*, *N. bruchi* (Curculionidae) and *Eccritotarsus catarinensis* (Miridae). Using these data, plant productivity and insect activity was modelled against fine-scale temperature data incorporating three distinct microclimates from 14 field sites distributed throughout South Africa's climatic regions.

Water hyacinth and its natural enemies were found to be negatively affected by low average temperatures. However, the relative consequences for each species at a population level were quite different. Similar thresholds for development, close to 10°C, meant that periods available for growth in areas where temperature is limiting were roughly the same for both plant and insects. Nevertheless, although plant growth largely ceased each winter and aerial parts were often extensively damaged from frost, low temperatures rarely led to significant plant mortality. By contrast, reduced insect

recruitment coupled with a high susceptibility to cold- and frost-induced mortality of all life-history stages, pushed insect populations into winter bottlenecks and even caused local extinctions. The ability to overwinter effectively appears to the primary cause for limited control in colder regions.

Surviving post-winter insect populations were therefore small, inflicted minimal damage due to reduced feeding rates, and were generally asynchronous with the recovery of water hyacinth. This asynchronous development translated into a lag period of roughly 42 days between the onset of water hyacinth growth and the time at which the plant was subjected to meaningful herbivory. Free from early season herbivory, coupled with the fact that vegetative reproduction continued through winter, water hyacinth populations were able to quickly recover and outpaced the detrimental affects caused by insect feeding well into the growth season. The implications for supplementary management strategies are also discussed in light of these outcomes.

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CHAPTER 1 – INTRODUCTION

1.1 Temperature-dependent development

The behaviour and physiology of every insect, during all developmental stages, is largely determined by temperature (Liu *et al*. 1995). Temperature influences an insect's metabolic rate (Addo-Bediako *et al*. 2002), flight activity, nutrition, growth rate (Lombardero *et al*. 2000), oviposition and longevity (McAvoy and Kok, 1999). Consequently, insect development occurs within a definite temperature range which can be experimentally determined (Wagner *et al*. 1984). Such studies on temperature-dependent development provide information on insect biology; contribute knowledge that enhances the efficiency of mass rearing; serve as a basis from which models that estimate insect growth, development and reproduction can be formulated (Zilahi-Balogh *et al*. 2003); and are useful in predicting field activity and phenology (Davis *et al*. 1996; Goolsby *et al*. 2005; Skinner *et al*. 2006). Additionally, differential survival ability at temperature extremes is a critical determinant of insect distribution limits which therefore closely follow climatic trends (Ungerer *et al*. 1999). Studies on temperature-dependent development are therefore important for understanding predator-prey relationships and insect population dynamics relevant in epidemiology, pest management and biological control of weeds and insect pests (Sutherst and Maywald, 1985).

In addition to a given climate, the physical environment or habitat, and various biological factors such as food selection, and both inter- and intraspecific competition, determines the presence or absence of an animal and influences its population size (Sutherst and Maywald, 1985). Ungerer *et al*. (1999) showed that the northern distribution limits of the North American timber pest *Dendroctonus frontalis* Zimmermann (Scolytidae) are maintained by the occurrence of lethal winter temperatures. By considering direct physiological measurements (lower lethal temperatures and developmental rates) it was found that temperature induced mortality was the sole barrier to northward range expansion. The presence of host trees and favourable summer temperature regimes farther north than the potential distribution limit would permit sufficient generations to sustain a viable population of the beetle. Consequently, northern distribution limits

fluctuated by several hundred kilometres depending on the recent history of winter temperatures. Thus, insect distribution boundaries can respond rapidly to variations in critical environmental variables (Cherrill and Begon, 1989; Ungerer *et al*. 1999).

Temperature regimes associated with distinct climatic regions also promote substantial variation in physiological traits between species and/or populations living in different areas (Chown *et al*. 2002; Klok and Chown, 2005). Insects show a wide variety of physiological traits at several spatial scales, ranging from microgeographic to macrophysiological (Klok and Chown, 2005). These traits include high and low temperature tolerance (Mitchell *et al*. 1993; Block *et al*. 1998), water balance characteristics (Sinclair *et al*. 2003) and developmental and metabolic rates (Wagner *et al*. 1984; Addo-Bediako *et al*. 2002). Using a global-scale analysis of 364 species, Addo-Bediako *et al*. (2002) showed that environmental temperature significantly influences the metabolic rate of insect species. Individuals from colder environments tended to have an elevated metabolism compared with those from warm climates. Whilst variation in physiological traits linked to thermal tolerance is fairly common in both inter- and intraspecific studies (Chown *et al*. 2002), this is not the rule. Klok and Chown (2005) have shown that at the population level, variation can be limited or mainly due to phenotypic plasticity. In a study on thermal tolerance limits and desiccation resistance in the moth *Embryonopis halticella* Eaton (Yponomeutidae), two sub-Antarctic populations were found to be physiologically similar despite living on separate, climatically dissimilar islands. The general lack of adaptive or 'predictable' variation in physiological traits makes the already intensive construction of models describing insect range limitation and population dynamics more difficult.

The principle of using temperature to describe an insect's life-history events has been recognised for more than 250 years (Higley *et al*. 1986). Phenological models estimate the proportions of a population residing in each life stage as a function of time and environmental conditions. Foremost of these conditions is temperature but other factors such as food source, humidity and solar radiation can significantly impact phenology. Recognising the timing and proportioning, or relative duration, of each of an insect's life

stages is essential for evaluating their ecological interactions. Food consumed, behavioural pattern and susceptibility to predation are all dependent on life stage (Stedinger *et al*. 1985; Chong *et al*. 2003). By providing insights into key events of an insect's life cycle, these models are important to researchers and land managers and have become established tools in botany, pest management, ecology (Higley *et al*. 1986), agricultural practice (Satake *et al*. 2006) and biological control (Skinner *et al*. 2006). Predicting events such as first emergence and peak abundance is essential for the scheduling of census samples, collection of biological control agents for redistribution, and control tactics such as burning, grazing and the application of herbicides or pesticides to maximise their effectiveness (Wagner *et al*. 1984; Satake *et al*. 2006; Skinner *et al*. 2006). Generation numbers can also be important in predicting invasion success and therefore serve as a basis for prioritising areas of concern for control (Pilkington and Hoddle, 2006). Accurate predictive phenological models require a thorough understanding of insects' thermal requirements for development (Wagner *et al*. 1984).

1.2 Phenological models

Phenological models are traditionally based on the assumption that the rate of insect development increases roughly linearly in response to increasing temperature (Wagner *et al*. 1984; Roltsch *et al*. 1999; Snyder *et al*. 1999). Furthermore, this development only occurs within a definite range between upper and lower temperature thresholds. At a basic level, development of an organism is reliant on the rates of biochemical or enzymatic reactions which depend on the availability of substrates, enzymes, and on temperature (Higley *et al*. 1986). At the lower temperature threshold, insect development approaches zero because this is the lowest temperature at which these biochemical reactions will occur. Under these circumstances insects often survive for sustained periods at cold temperatures with little or no development. As temperatures increases beyond the lower threshold, developmental rate becomes proportional to temperature but will slow as the optimum temperature is reached and drop sharply as the upper threshold is approached (Wagner *et al*. 1984). The upper developmental threshold occurs at a temperature where kinetic constraints limit enzyme activity and substrate diffusion. Beyond this, temperatures become high enough to produce structural changes in control

enzymes, impair enzyme function or actually denature some proteins. Consequently, the upper threshold often approaches the lethal limit of a species (Higley *et al*. 1986). The most popular approach, incorporating this linear response to temperature into usable predictive models, is that of heat unit or degree-day accumulation (Roltsch *et al*. 1999).

Phenological models have been used extensively as decision support tools for improving the efficacy of pest management (Zalom *et al*. 1983; Herms, 2004). Many examples exist in the literature, ranging from the prediction of disease susceptibility in plants (Danneberger and Vargas, 1984), to improved forecasting of insect life-history events, useful for the scheduling of control tactics (Davis *et al*. 1996; Satake *et al*. 2006). Chong *et al*. (2003) demonstrated how accurate forecasting can improve pest management. The madeira mealybug, *Phenacoccus madeirensis* Green (Pseudococcidae), is a serious pest in greenhouse ornamental production requiring regular chemical applications to suppress an infestation. Immature stages must be targeted as the adult mealybugs are difficult to control because of a thick waxy secretion surrounding the body. At lower temperatures, the immature stages were shown to have a longer duration of development resulting in a broader window of opportunity for management via chemical application. Manipulation of the temperature within the greenhouse therefore, could facilitate better management of the pest.

Phenological models are also increasingly applied to beneficial insects, such as those utilised within biological control systems; and have proved useful for new agent selection (Geden, 1997; Hoelmer and Kirk, 2005), in predicting establishment (McClay and Hughes, 1995; Pilkington and Hoddle, 2006), and potential efficacy (Stewart *et al*. 1996; Skirvin and Fenlon, 2003). Additionally, temperature dependent models have been used to evaluate natural enemies in terms of population synchrony and have facilitated the incorporation of biological control into a variety of integrated management strategies (Roy *et al*. 2005). Leafy Spurge, *Euphorbia esula* L., is an invasive of rangelands in the upper Great Plains of the United States. Management of the weed using chemical, cultural and mechanical removal has proved unsustainable in the long-term and it has consequently been targeted for biological control. Using accumulated degree-days,

Skinner *et al*. (2006) showed how the prediction of peak emergence of two flea beetles, *Aphthona lacertosa* Rosenhauer and *A. nigriscutis* Foudras (Chrysomelidae) could aid integrated control of leafy spurge. As flea beetles pupate in the soil, the timing of burning regimes prior to beetle emergence allowed land managers to control other weeds without affecting natural enemy populations. Similarly, sheep allowed to graze on leafy spurge until beetle emergence also limited weed populations. Furthermore, collecting beetles for redistribution prior to peak abundance when egg loads are still high would result in a higher potential for establishment at new localities (Skinner *et al*. 2006). This type of integrated approach is especially relevant in cold climates where seasonality and low temperatures can limit the efficacy of management strategies.

1.3 Biological control in cool climates: A call for integrated management

The Rapoport effect has become widely acknowledged for explaining high species richness in the tropics. It states that the extent of a species' latitudinal distributions increases with an increase in latitude (Addo-Bediako *et al*. 2000). This has also been extended to encompass similar altitudinal changes in species richness (Chown *et al*. 2002). The principle mechanism proposed to explain these trends is the fact that to survive at higher latitudes and altitudes an organism needs to be able to withstand greater temporal variability in climatic conditions. Consequently, species that have evolved at higher latitudes or altitudes have wider physiological tolerances than those near the tropics or at lower altitudes (Addo-Bediako *et al*. 2000; Chown *et al*. 2002). This does not bode well for biological control in temperate climates where natural enemies introduced from the tropics often lack the suite of behavioural and physiological adaptations necessary to cope with lower temperature environments.

Temperature minima are often cited as a limiting factor for the successful establishment of biological control agents into novel climates (McClay, 1996; McEvoy and Coombs, 2000). In situations where successful establishment has taken place, failure to achieve adequate control is frequently attributed to incompatibility with local climatic conditions in the area of introduction (Stewart *et al*. 1996; Byrne *et al*. 2002). McClay (1996) reports on two biological control programmes in Alberta, Canada, the most northerly region in

which classical biological control has been practiced. Climatic conditions here are characterised by short growing seasons and extremely long winters. The establishment of the moth *Calophasia lunula* Hufnagel (Noctuidae) introduced against toadflax (*Linaria* spp.) was found to be limited by low temperatures and short growth seasons preventing the completion of a single generation. By using a Degree-day model, McClay and Hughes (1995) showed that the accumulation of heat units in central and northern Alberta was well below what is required for *C. lunula* development. This model subsequently led to the termination of further releases of *C. lunula* allowing efforts to be concentrated elsewhere. In a second programme, McClay (1996) showed that although the chrysomelid beetle *Lema cyanella* L., introduced against Canada thistle (*Cirsium arvense* (L.) Scop.), does establish in Alberta, its rate of population increase is limited by low fecundity in cool conditions. It is evident therefore, that low temperatures are capable of limiting the effectiveness of biological control programmes.

Cruttwell McFadyen (1998) states that successful biological control of weeds depends on three main criteria. The first two relate to the natural enemy itself: the relative amount of damage the agent can inflict on a target weed; and the agent's ecology which determines the population density achieved in the new environment. In the case of *L. cyanella*, slow population increase translates into small populations only able to inflict limited damage on *C. arvense* infestations. The third criterion relies on the ecology of the target weed, which will determine whether the total damage is significant in reducing its population. Differing natural enemy and target weed ecologies, brought on for whatever reason, can lead to seasonal asynchrony between the organism's life histories.

Synchrony of population dynamics has been relatively well addressed in agricultural pest scenarios, either in biological control programmes against agricultural pests or directly in plant-pests themselves. Roy *et al*. (2005) showed that early phenological synchrony between the mite *Tetranychus mcdanieli* McGregor (Tetranychidae), a pest of raspberry, and its two predators was a critical factor underlying its successful control. Within biological control of arthropod pests, Roy *et al*. (2005) maintain that asynchrony can result from differences in temperature tolerances between natural enemies and their hosts,

allowing pests to escape early season regulation and to reach damaging levels. However, in weed biological control, few examples exist which describe the effects of temperature on insect/host synchrony. Dynamics analogous to those prevalent in weed control systems are evident from a study by Satake *et al*. (2006) describing the population dynamics of a tea pest. In this case, asynchrony between plant phenology and the temperature controlled timing of adult emergence of the tea leaf roller *Caloptilia theivora* Walsingham (Gracillariinae) reduced the amount of damage to the crop. Although not solely brought on by temperature, Müller *et al*. (1990) describes an example of natural enemy and host-plant asynchrony contributing to the failure of biological control of russian thistle *Salsola australis* R. Brown (Chenopodiaceae). In this case, the oviposition period of the moth *Coleophora parthenica* Meyrick (Coleophoridae) coincided with the highest mortality of young host plants brought on by limited soil water. The resultant decrease of moth density between the overwintered and first generation was interpreted as a direct result of poor host-plant synchrony caused by the differential impact that climatic conditions had on plant and moth populations.

The establishment and efficacy of introduced natural enemies into cooler climates may thus be limited by low temperatures preventing rapid population increase; promoting high over-wintering mortality (Stewart *et al*. 1996), and natural enemy and host-plant asynchrony (Müller *et al*. 1990); slowing development and feeding rates (Chown and Nicolson, 2004) or inhibiting processes such as oviposition (McClay, 1996). In these circumstances, as in other ineffective biological control systems, emphasis has traditionally focused on acquiring additional natural enemies to supplement control. This practice however often creates needless complexity, redundancy, and "increases the risk in an already risky business" (McEvoy and Coombs, 2000; Thomas and Willis, 1998). More recently, views are moving away from this 'lottery approach' towards more integrated management strategies (McEvoy and Coombs, 2000; McCoy and Frank, 2010). Integrated weed management is aimed at optimizing and economising long-term mechanical, cultural and chemical control of invasive weeds. Wherever possible, this optimization is done in harmony with the environment, and in so doing, emphasises the importance of biological control. Few studies however have attempted or reported the

research needed to support decision making as well as determine how these tactics work together in practice (McEvoy and Coombs, 2000).

Integrating control techniques is especially relevant for the control of invasive weeds in South Africa (Zimmermann and Neser, 1999). South Africa is extremely variable in terms of climate, including high-altitude regions subjected to cold, dry winters with frequent frosts, Mediterranean winter rainfall areas where frost is absent, and coastal subtropical summer rainfall areas (Schulze *et al*. 1997). These conditions have limited natural enemy efficacy (Hill and Olckers, 2001) as well as hampering the establishment of others (Byrne *et al*. 2002) and have highlighted a need for supplementary control measures. Consequently, integrated management strategies have become increasingly prominent in tackling many of South Africa's worst weeds (Zimmermann and Neser, 1999), including water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) (Jones, 2001; Van Wyk and Van Wilgen, 2002).

1.4 Water hyacinth

Water hyacinth is frequently branded as the world's worst aquatic weed due to its invasive potential, negative impact on aquatic ecosystems and the cost it necessitates to control it (Gopal, 1987; Julien *et al*. 1996; Van Wyk and Van Wilgen, 2002). Originally a native of the Amazon basin, the weed has spread throughout tropical, subtropical and some warmer temperate regions around the world since the late 1800s (Julien *et al*. 1996). The present day distribution is largely due to the activities of man who has served as the chief agent of dispersal. High seed production, its free-floating habit and rapid growth and population increase via vegetative reproduction, then allows the weed to become both widespread and firmly entrenched in areas of introduction (Gopal, 1987). Vegetative growth rates are very high under ideal conditions, which can be up to 6% per day (Ashton *et al*. 1979). Edwards and Musil (1975) reported that populations of the weed can double their numbers every 11 to 18 days through vegetative growth alone under optimum conditions.

Accounts of different growth forms of *E. crassipes* appear in the literature, highlighting the variable morphology of the plant (Penfound and Earle, 1948; Center and Spencer, 1981). Water hyacinth is typified as a perennial, herbaceous, free floating aquatic plant with erect aerial leaves and submerged roots. The plant is composed of a sympodially branched, stoloniferous rhizome up to 30cm in length, which produces a rosette of petiolate leaves arranged in a whorl. Petioles of free floating individual plants are short, spongy and form a bulbous 'float'. Once growth is sufficient to cause crowding of individual plants, extensive interweaving of parts occurs, forming a dense self supporting mat. In these situations, petioles elongate, generally up to 30-50cm in length, and cease to function as 'floats'. The roots are adventitious, fibrous, unbranched, have a conspicuous root cap and produce numerous laterals of limited length, giving them a fine feathery appearance (Center and Spencer, 1981; Gopal, 1987). Root growth is extensive, Center and Spencer (1981) cite a study which found the combined length of 158 roots and their laterals from an average sized plant to be 11 481m, with a total area of 7.31m^2.

Water hyacinth produces numerous lilac flowers borne on terminal inflorescences. Eight to 15 flowers per inflorescence is common, although as many as 35 have been recorded, each producing as much as 450 mature seeds (Gopal, 1987). Each inflorescence therefore seldomly produces less than 3000 seeds, and a single plant can produce several inflorescences per year. These seeds have been estimated to remain viable for 15-20 years (Edwards and Musil, 1975; Gopal, 1987), readily germinate in moist sediments or shallow warm water, and flowering can commence 10-15 weeks thereafter. Water hyacinth seeds however, require very shallow water with high light intensities and warm temperatures for germination and consequently in most situations, seedling recruitment is limited by the lack of suitable germination sites. Edwards and Musil (1975) reported successful germination with a mid-day water temperature of 28-36°C, and air temperature between 21°C and 27°C. Therefore, population increases of *E. crassipes* at heavily infested sites are predominantly facilitated by vegetative reproduction whereas re-infestation of cleared areas occurs via propagation by seeds (Van Wyk and Van Wilgen, 2002). Vegetatively produced plants or ramets are formed from auxiliary buds on stolons produced through the elongation of internodes (Center and Spencer, 1981).

1.5 Managing water hyacinth in South Africa

Eichhornia crassipes was first recorded in South Africa on the Cape flats in the early 1900s. Since then, nutrient enriched waters have facilitated its spread throughout the country severely degrading aquatic ecosystems and limiting their utilization (Hill and Cilliers, 1999). These negative effects include the suppression of indigenous aquatic fauna and flora (Jones, 2009), the obstruction of river flows which may aggravate flooding and promote siltation, and increased rates of evapotranspiration from water storages. Edwards and Musil (1975) report that water hyacinth cover increases water loss to the atmosphere 3.2-3.7 times that of a free water surface via evapotranspiration. Water hyacinth infestation also poses a potential health risk in that the plant has been implicated in the creation of breeding habitats for malaria carrying mosquito larvae as well as other disease vectors such as the bilharzia snail (Edwards and Musil, 1975).

Combating water hyacinth infestations has drawn upon varied sources of management techniques designed to reduce both the weeds spread and biomass. These include physical removal, application of herbicides, utilisation for commercial and subsistence purposes and the importation and release of biological control agents. In most cases however, the use of physical or chemical control techniques in isolation are both costly and unsustainable in the long-term (Julien *et al*. 1996; Van Wyk and Van Wilgen, 2002). Water hyacinth can be utilized in a variety of ways at a subsistence level including use as a fertilizer, fodder, fish feed, paper and fibre source, or for mineral nutrient removal from polluted water, and to power alcohol and biogas production (Malik, 2007). Eradicating it can therefore create a potential conflict of interest. However, Julien (2001) stresses that these uses seldom develop into sustainable activities and the cost of water hyacinth to communities far outweighs any benefits derived through utilization. Biological control remains the most cost-effective and environmentally friendly technique for the sustainable control of water hyacinth (Julien *et al*. 1996; Julien, 2001; Van Wyk and Van Wilgen, 2002).

Since the 1970's water hyacinth control in South Africa has largely depended on the use of herbicides, while biological and integrated control has received far less effort and investment. Despite this, a biological control programme was initiated against the weed in 1973 but was terminated in 1975 due to interference from uncoordinated herbicide control operations. Biological control was restarted in 1985 after a request from the Department of Water Affairs and Forestry to develop an integrated management plan for the weed (Van Wyk and Van Wilgen, 2002).

1.6 Biological control of water hyacinth

The biological control of *E. crassipes* in South Africa currently relies on six established agents, the largest number in all of the countries involved in such programmes against water hyacinth. In 1973, the weevil *Neochetina eichhorniae* Warner (Curculionidae) was released in South Africa, and re-released in 1985 when biological control was resumed. By 1990, *N. eichhorniae* had become widespread throughout the country but had only limited effect on the weed (Hill and Cilliers, 1999). For this reason, it was deemed that *N. eichhorniae* would be unable to achieve the desired level of control on its own and additional natural enemies were sought. Subsequently, a second weevil, *Neochetina bruchi* Hustache, a pyralid moth, *Niphograpta albiguttalis* Warren (Pyralidae), and the water hyacinth bug *Eccritotarsus catarinensis* Carvahlo (Miridae) were released and have become established. In addition to these, the galumnid mite, *Orthogalumna terebrantis* Wallwork (Galumnidae), as well as a fungal pathogen, *Cercospora piaropi* Tharp (Hyphomycetes), which were never released, have become widely established at many localities but with minimal impact (Hill and Cilliers, 1999; Van Wyk and Van Wilgen, 2002).

The South American weevil *N. eichhorniae* is the most abundant and widespread of the natural enemies on water hyacinth in South Africa. Despite this, it has only marginally controlled the weed in certain regions, for example, in informal biological control reserve sites along the Vaal and Nseleni Rivers, and in New Years Dam in the Eastern Cape Province (Hill and Cilliers, 1999; Jones, 2001). Releases at the latter in 1990 resulted in an 80% reduction in weed mat cover by 1995. Subsequent releases of *N. bruchi* in 1996,

coupled with the already established population of *N. eichhorniae* maintain cover on the dam at a fluctuating level between 10% and 80% (Van Wyk and Van Wilgen, 2002). It has tentatively been suggested that *N. bruchi* is better able to damage water hyacinth growing in nutrient enriched waters (Heard and Winterton, 2000), and performs better at colder temperatures (DeLoach and Cordo, 1976a) when compared to *N. eichhorniae*. Consequently, since 1996, emphasis has shifted to rearing and releasing *N. bruchi* in South Africa (Hill and Cilliers, 1999), as well as fully implementing all of these agents around the country.

Niphograpta albiguttalis was first introduced from South America in 1990 but has had only a limited impact on local water hyacinth infestations due to considerable variation in its population densities both spatially and temporally (Oberholzer, pers. comm.). In six years of observations at several sites in Argentina, DeLoach and Cordo (1978) found that populations of the moth varied extensively between years and between sites in the same year. The moth typically targets the colonizing form of water hyacinth and thus, damage is concentrated on young, vigorously growing plants with bulbous petioles (DeLoach and Cordo, 1978; Hill and Cilliers, 1999). This preference means that the moth is most effective on new infestations, which is assumed to lead to its correspondingly patchy distribution around the country. Despite this, *N. albiguttalis* remains an integral part of the control of water hyacinth as it complements the effectiveness of the two weevils (DeLoach and Cordo, 1978) and has been cited as having a broad climatic tolerance and therefore able to withstand the severe winters of high altitude regions of the country (Hill and Cilliers, 1999). Additionally, the moth's preference for younger, 'colonizing' plants has highlighted its use within an integrated system where water hyacinth infestations can be 'thinned' using herbicides (Jadhav *et al*. 2008; Byrne *et al*. 2010).

Orthogalumna terebrantis has become well established in warmer areas of South Africa where it reaches extremely high population densities resulting in extensive damage to infested water hyacinth leaves (Hill and Cilliers, 1999). Although the mite has failed to establish at a number of high altitude sites, for example on the Vaal River, Hill and Cilliers (1999) speculate that this is not due to poor low temperature tolerance. Cordo and

Deloach (1976) showed that the mite was tolerant of temperatures as low as -10ºC and more importantly, could overwinter in all stages of development. Indeed, its native distribution in South America appears to be limited in the south by the presence of water hyacinth and not constrained by climate (Hill and Cilliers, 1999). The mite's ability to withstand cold temperatures for prolonged periods can be seen as an obvious advantage for future biological control initiatives in colder regions of South Africa.

Eccritotarsus catarinensis, originating on the east coast of Brazil, is the most recent natural enemy released against water hyacinth anywhere in the world. It was released in South Africa in 1996 but has had a limited impact on water hyacinth infestations, and has failed to establish at a number of localities (Hill and Cilliers, 1999). Despite this, the mirid was anticipated to be highly damaging given its high intrinsic rate of natural population increase. Nevertheless, subsequent investigation indicated that the efficacy of *E. catarinensis* in South Africa would be limited by low temperatures (Coetzee *et al*. 2007b) and that herbivory from the mirid in isolation would not be sufficient to reduce all aspects of water hyacinth vigour, especially when grown under high nutrient conditions (Coetzee *et al*. 2007a).

In general, the results of the biological control programme in South Africa do not compare with the achievements made elsewhere, such as in the tropical and sub-tropical systems in Central Africa (Julien, 2001; Wilson *et al*. 2007). The lack of success in South Africa has been attributed to a number of constraining factors such as a wide variety of climatic regions, low minimum temperatures and a high incidence of frosting which slows the build-up of natural enemy populations. Other factors include high rates of water hyacinth growth stimulated by nutrient enriched waters, and the regular removal of water hyacinth infestations by flooding, herbicide application and mechanical clearing, which results in a loss of natural enemy habitat and food source (Hill and Olckers, 2001; Van Wyk and Van Wilgen, 2002). Considering the world-wide occurrence of water hyacinth, the weed's distribution within South Africa is unlikely to be limited by temperature. Adequate control of water hyacinth will therefore only be accomplished if the effects of temperature and the control of nutrient inputs into waterways forms an integral part of

any future management strategy. Consideration of these factors will also assist in the prediction of relevant biological events related to plant and insect phenology, thus enabling accurate timing of other supplementary management strategies, such as herbicide sprays, in order to maximise their efficiency.

1.7 Focus of this research

Cruttwell McFadyen (1998) maintains that the evaluation of biological control programmes is essential to justify continued expenditure. Although the programme initiated against water hyacinth in South Africa has received continued attention since its inception over 30 years ago, certain control tactics have been prioritised without adequate substantiating research. For example, the mass rearing and re-releasing of potentially ineffective agents has continued despite their realised efficacy in field situations being unknown, as well as numerous and repeated failures to establish. Herbicides have also been used extensively without adequate consideration given to optimising spray volumes or dosages, and timing. These scenarios are most often costly, allow the problem to get worse when time is wasted waiting for a result and have the potential to undermine other control strategies. Prioritising different control tactics and suites of control tactics on a site by site basis is therefore paramount to controlling water hyacinth in South Africa. Although examples of successful management of water hyacinth using an integrated approach exist (Jones, 2001), the change in mindset of biological control practitioners and land managers has been slow and little research has been done supporting any future country wide integrated management plan.

As described above, climate and indeed temperature is one of the primary abiotic factors controlling insect behaviour and physiology and one of the key determinants of plant phenology. Understanding the thermal tolerance of water hyacinth and its introduced natural enemies is essential for developing predictive models of their phenology, efficacy and potential for establishment in a variety of field scenarios. This research will therefore attempt to verify the thermal tolerance data accumulated on three of the more widespread and effective species released against water hyacinth in South Africa. Additionally, because low temperatures restrict natural enemy efficacy by adversely affecting plant

growth, existing cold tolerance data on water hyacinth will be augmented and placed in a South African context. Plant productivity and insect performance will be correlated to temperature by investigating the effects of long term exposure of the plant and its natural enemies to low, yet ecologically relevant temperatures. Knowledge of how these temperatures affect plant productivity and arthropod performance will allow prediction of population growth rates, phenology and likely agent efficacy.

From a model incorporating stage structured density dependent development of *N. eichhorniae*, Wilson *et al*. (2006) predicts that the main factor limiting biological control of water hyacinth in field situations appears to be the developmental rate of the weevil relative to the plant and its seasonal phenology. This suggests that density dependent regulation of the early weevil instars will slow responses to changes in the plant population and delay the population build-up of the insects. In this study, field monitoring will be used to determine whether or not this lag period between plant and natural enemy population build-up exists, whether it is accentuated in situations with lower average temperatures, under which temperature regimes this lag period fluctuates, and to construct the seasonal phenology of both the plant and its natural enemies under different climatic conditions using degree-day models. These models will allow forecasts of natural enemy population growth, survival, and life-history stage present, relative to plant population growth or biomass accumulation for different temperature regimes around the country over time. Accurate prediction of these criteria is especially important and should be given careful consideration when integrating control measures. For example, because density dependence in early instar larvae is a crucial factor in regulating *N. eichhorniae* populations, Wilson *et al*. (2006) maintains that the weevils will be sensitive to changes in leaf dynamics. Therefore, other control options such as herbicide spraying could have the potential to seriously undermine the control facilitated by the weevils if mistimed. This research will enable climatic factors to be incorporated with nutrients and herbicide application into an integrated management plan for the sustainable control of water hyacinth throughout South Africa.

1.8 Research aims

To assess the cold tolerance of water hyacinth and its natural enemies.

- An assessment of the acute effects of low temperature and frost on plant growth and reproductive output.
- Calculate the degree-day thresholds for *N. eichhorniae*, *N. bruchi*, and *E. catarinensis* from existing literature.

To quantify the long-term or chronic effects that low temperatures have on the productivity of water hyacinth and the development and efficacy of its natural enemies.

- Evaluate insect performance based on feeding rates, fecundity and mortality relative to low fluctuating temperatures.
- Evaluate plant productivity based on growth rates and reproductive output relative to low fluctuating temperatures.

To accurately describe the seasonal phenology of water hyacinth and its natural enemies under South African field conditions.

- Construct a country wide phenology based on monthly field measures and continuous temperature monitoring.
- Combine the above data in order to construct degree-day models describing key events in the population dynamics of *N. eichhorniae*, *N. bruchi*, and *E. catarinensis* relevant to South African conditions.

CHAPTER 2 – CORRELATING PLANT PRODUCTIVITY AND INSECT PERFORMANCE WITH TEMPERATURE

2.1 Introduction

2.1.1 Impact of low temperature on biological control of water hyacinth

Highly eutrophic waters and minimum temperature extremes are emphasised most often in the literature as factors which limit the impact of the biological control agents released against water hyacinth in South Africa (Hill and Olckers, 2001; Coetzee *et al*. 2007b; Byrne *et al*. 2010). Various authors speculate that waters enriched with nitrates and phosphates enable water hyacinth growth to outpace any detrimental effects inflicted by their natural enemies (Reddy *et al*. 1989; 1990). Additionally, Van der Heide *et al*. (2006) maintain that the impact of herbivore grazing is not only dependent on the feeding rate of the insects, but also on the growth rate of the plants involved, both of which are differently affected by temperature. Possible asynchrony of population growth brought on by different responses of water hyacinth and its natural enemies to low temperatures will compound these problems, further limiting control, especially in colder areas.

Cold climates are assumed to limit control in two additional ways: cooler average temperatures may hamper the efficacy of natural enemies by slowing development and consequently population growth rates, or directly by causing mortality. Winter minimums in high-altitude interior regions of South Africa frequently drop below physiologically significant temperatures, for example, the temperatures at which development is arrested in both *N. eichhorniae* and *N. bruchi* weevils and *E. catarinensis* (Table 2.1). Secondly, because water hyacinth is adversely affected by low temperature extremes, natural enemy survival is thought to be further limited by habitat destruction and food availability. Grodowitz *et al*. (1991) reported declines in living plant biomass, the number of leaves per plant, and plant density, associated with winter at a Texas location in the USA. Declines in the number of *N. eichhorniae* pupae and adults were also found to coincide with this period. Aerial parts of water hyacinth brown and die back, and crowns submerge when exposed to low temperatures and frost (Owens and Madsen 1995). This loss of habitat, food supply and decreased nutritive status of plant tissue is anticipated to

lead to an increase in mortality of natural enemies overwintering on aerial parts of the plant (Grodowitz *et al*. 1991). Frost has been shown to influence the geographical distribution of some insect species and has even been implicated in causing local extinctions (Inouye, 2000). Ehrlich *et al*. (1972) describe the local extinction of the butterfly *Glaucopsyche lygdamus* Doubleday (Plebejinae), caused by the loss of host plants as a direct result of heavy frosting.

The low temperature limits of water hyacinth were investigated by Owens and Madsen (1995). Regrowth experiments in field and laboratory conditions were conducted on the ability of water hyacinth to survive low air temperatures. Subjecting plants to low temperature extremes for short periods of time in the laboratory, exposure to -16ºC for

Table 2.1. Thermal tolerance data for the biological control agents *Neochetina eichhorniae*, *Neochetina bruchi* and *Eccritotarsus catarinensis* released against water hyacinth in South Africa. Source: 1. Coetzee *et al*. 2007b; 2. Coetzee, unpub.; 3. DeLoach and Cordo, 1976a; 4. Julien, 2001.

	Neochetina bruchi	*Neochetina eichhorniae*	*Eccritotarsus catarinensis*
Lower LT_{50}	-	-7.4°C [2]	-3.5°C [1]
Upper LT_{50}	41.6°C [2]	41.7°C [2]	37.0°C [1]
CT_{min}	3.3°C [2]	4.3°C [2]	1.2 ± 1.2°C [1]
CT_{max}	48.8°C [2]	51.0°C [2]	49.6 ± 3.4°C [1]
Lower oviposition threshold	-	10°C [3]	-
Lower developmental threshold	≈15°C [4]	-	10.3°C [1]
Degree-day requirements (egg to adult)	-	-	342°D [1]
Optimum temperature for feeding/ oviposition	30°C [4]	30°C [4]	-

less than 12 hours was found not to produce any significant stem base mortality. Placing this into a South African context, air temperatures never get cold enough, for long enough, to cause stem base mortality in the field. Longer term experiments with mean minimums revealed that exposure to constant temperatures below 5ºC for at least two weeks were required to cause stem base mortality (Owens and Madsen, 1995), an equally unlikely scenario in South African ecosystems. These findings are useful in that unlike its biological control agents, water hyacinth survival will seldom, if ever, be restricted by temperature anywhere in South Africa. However, Owens and Madsen's (1995) treatment plants had all aerial parts removed, leaving only stem bases with attached roots. This is unfortunate as the results give no indication of the temperature range at which leaf mortality occurs, or when plant vigour is reduced. As the loss of leaf material is linked to natural enemy survival, and leaf mortality is prevalent throughout much of the interior regions of South Africa (Van Wyk and Van Wilgen, 2002), this temperature range would indicate periods of plant dormancy, reduced growth rate, and leaf mortality, and could serve as a valuable predictor of potential for establishment the mirid and mite, which persist and feed on aerial parts of the plant. Understanding how temperature affects water hyacinth and indirectly, its natural enemies is only half the story. Nevertheless, models, of varying complexities, have been developed in order to understand how temperature affects insects directly (E.g. Campbell *et al*. 1974; Lactin *et al*. 1995; Ikemoto and Takai, 2000).

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

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

$$y = a + bT$$

where y is the rate of development (1/Days), and T is the temperature (°C) at which the insect was reared, remains one of the most widely used methods for estimating insect

development due to its simplicity and relative accuracy. However, this method is less effective when considering smaller data sets that may include developmental rates at extreme temperatures that would certainly lie off the linear portion of this line (Bonhomme, 2000). Ikemoto and Takai (2000) described three drawbacks associated with this method. Firstly, as there is an optimum range of temperatures for which an insect's development is roughly linear and which is constrained between specific upper and lower temperatures, a regression line should only be applied to this portion of development. The detection of these critical upper and lower temperatures, outside of which development deviates from the linear trend, is difficult and open to bias when using the regression method. This results in unreliable estimations of the developmental threshold (*t*), the temperature below which an organism's development is arrested, and the degree-day requirements or thermal constant (K), the duration of development from egg to adult relative to temperature. Secondly, a simple regression assumes constant variances on the 1/Days scale at all temperatures resulting in the disproportionate weighting of data points in the upper and lower sections of the line, being more exaggerated at the lower temperature range. Thirdly, the regression line ignores variation in temperature. The second and third problems would result in an underestimation of *t* and a larger estimation of K. To overcome these problems Ikemoto and Takai (2000) propose a different line-fitting method.

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

$$(DT) = K + tD$$

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

developmental thresholds and thermal constants for both *N. eichhorniae* and *N. bruchi* are calculated using data derived from existing literature. These data points enable the amount of environmental heat available for insect development to be quantified, the units of which are known as degree-days. Degree-days and their application for phenological models, are employed later in this chapter and in chapter 3. A long-term evaluation of the effects of low temperature and frost on the growth of water hyacinth is also undertaken in this chapter. Leaf turnover rates and leaf mortality in response to temperature is investigated at three high-altitude or highveld sites prone to frequent winter frost for an 18 week period moving into winter.

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

asynchrony exists between the seasonal phenology of each by modelling their relative post-winter recovery, a key stage implicated as causing limited control in the colder high-altitude areas of South Africa (Hill and Olckers, 2001).

2.2 Materials and Methods

2.2.1 Temperature dependent development of *Neochetina bruchi* and *Neochetina eichhorniae*

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

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

$$SE_t = \frac{\bar{y}}{b}\sqrt{\frac{S^2}{N\bar{y}^2} + \left[\frac{SEb}{b}\right]^2} \qquad \text{and:} \qquad SE_K = \frac{SE_b}{b}$$

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

In light of the problems associated with the linear intercept method, the reduced major axis regression proposed by Ikemoto and Takai (2000) was also used. This allowed the parameters calculated from each method to be compared and the more realistic estimates used. Upon fitting the straight line x = D on y = DT to the data, the optimum temperature range was determined by excluding data points at higher and lower extreme temperatures which deviated from the linear tendency defined by the points in the optimum temperature range. A reduced major axis regression was then applied to this optimised temperature range in order to estimate the parameters t and K, determined from the line equation. Due to the limited developmental dataset available from existing literature, and missing data points for *N. eichhorniae* pupal development, data for both weevil species were combined and reanalysed using both the linear intercept and reduced major axis regression methods. Although not species-specific, these estimates of t and K are more applicable to field scenarios as populations of the two species are normally mixed and immatures cannot be readily differentiated.

2.2.2 Assessment of the effects of low temperature and frost on water hyacinth

Field observations of the prolonged effects of low temperature and frost on water hyacinth were done over an 18 week period from 28/03/2006 to 31/07/2006 at three highveld sites that receive regular frosting during winter. A 4.9 m^2 plastic lined wire mesh pool on the University of the Witwatersrand campus (26°11'29.68"S 28°01'56.94"E) and two approximately 3 000m^2 dams at Delta Park (26°07'29.28"S 28°00'39.52"E), the lower of which, is more heavily frosted due to the parks topography (Lockwood, pers. comm.), were used. As the surface of each system was 100% covered with water hyacinth, which limited plant movement and more crucially, determined similar plant architecture, sites remained comparable. The ratio of alive to dead leaves per plant, leaf production, number of ramets, longest petiole length and the severity of frost damage on 10 tagged plants at each site was recorded per week. Frost damage was categorised by ranking the amount of dead leaf tissue into one of six categories: 0%;

<5%; 5-25%; 25-50%, 50-75% and 75-100%. These measures were correlated to 'continuous' (every 30 minutes) microclimate monitoring facilitated by Thermochron iButtons (temperature range: -40°C to 85°C in 1°C increments with an accuracy of ±1°C). Water temperature 8cm below the surface, air temperature within the water hyacinth canopy and ambient air temperature 1.2m above the water surface was recorded. Canopy temperatures were adjusted to minimise errors associated with radiative heating of the temperature buttons (see chapter 3). The rate of leaf production per week was then regressed against the corresponding mean weekly water temperature. By grouping these data from the three sites, a standard curve describing the rate of leaf production in response to increasing water temperature was constructed.

2.2.3 Evaluation of insect performance and plant productivity in response to temperature

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

Each environmental chamber housed a pool containing 30 water hyacinth plants on which 2 pairs of adult weevils per plant, and 12 plants on which 10 adult mirids per plant were released respectively for both treatment and control. Water nutrient concentrations approximated intermediate levels observed in waters around South Africa (2 mg N l^{-1}; 0.29 mg P l^{-1}) (Brudvig *et al*. 2005) and were standard throughout all treatments, for both temperature regimes, and for all agents. Plants were maintained under wide spectrum fluorescent plant growth tubes. Plants and insects were left to acclimate for a week before

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

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

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

any observations were made. At the onset of weekly measures, plant productivity was gauged via counts of ramets, flowers and leaves per plant. Growth parameters recorded the plants phenostage, the length of longest and second youngest petiole, maximum root length, and the area of the second youngest leaf. For the duration of the weevil experiment, two plants were removed weekly from each temperature regime and destructively sampled. As the weevils prefer to feed on younger leaves – the number of feeding scars on the 2nd youngest leaf (DeLoach and Cordo, 1976a) and the number of eggs laid on the 3rd youngest leaf were recorded. Observations also recorded the number of larvae, pupae and adults present per plant removed. Adult insects found in excess of what was initially released per plant were released back onto their respective pools to ensure constant population densities.

Non-destructive sampling was performed weekly for the duration of the mirid experiment on three randomly selected plants; resampling of which was only done once all initial 12 plants had been sampled. Recorded parameters included the number of adults and nymphs per plant, and the leaf area damaged by mirid feeding on the 1st, 2nd and 3rd youngest leaves. Feeding was categorised by ranking the area of leaf tissue damaged into one of six categories: 0%; <5%; 5-25%; 25-50%, 50-75% and 75-100%. These measures were correlated to 'continuous' (every 30 minutes) temperature monitoring facilitated by Thermochron iButtons. Water temperature 8cm below the surface, air temperature within the water hyacinth canopy and ambient air temperature 1.2m above the water surface was recorded.

Constant temperature experiments were also run to assess the effects of temperature on the rate of adult *N. eichhorniae* feeding. 10 Adult weevils were released into Petri dishes containing cut water hyacinth leaves and kept at a constant temperature for between 24 and 72 hours. Severed petioles were wrapped in paper towel and kept moist in order to preserve leaf quality. Constant temperature rooms, controlling air temperature and humidity, with a 12 hour diurnal cycle, maintained the weevils at the prescribed experimental temperatures of 5°C, 12°C, 15°C, and 20°C respectively. Scar production per individual adult weevil was then calculated per 24 hour period.

2.3 Results

2.3.1 Temperature dependent development of *Neochetina bruchi* and *Neochetina eichhorniae*

Large discrepancies were found in the estimates for *t* and K by the different methods of Campbell *et al*. (1974) and Ikemoto and Takai (2000). The linear intercept method (Fig. 2.1) returned unrealistically low developmental thresholds for both weevil species, 2.9°C and 3.3°C for *N. bruchi* and *N. eichhorniae* respectively (Table 2.3). In line with the problems described by Ikemoto and Takai (2000), the linear intercept method also returned very large estimates of the thermal constants for both weevils, up to 1000 degree-days higher than expected when compared with other weevils living on aquatic plants (E.g. Mazzei *et al*. 1999). The reduced major axis regression technique (Fig. 2.2)

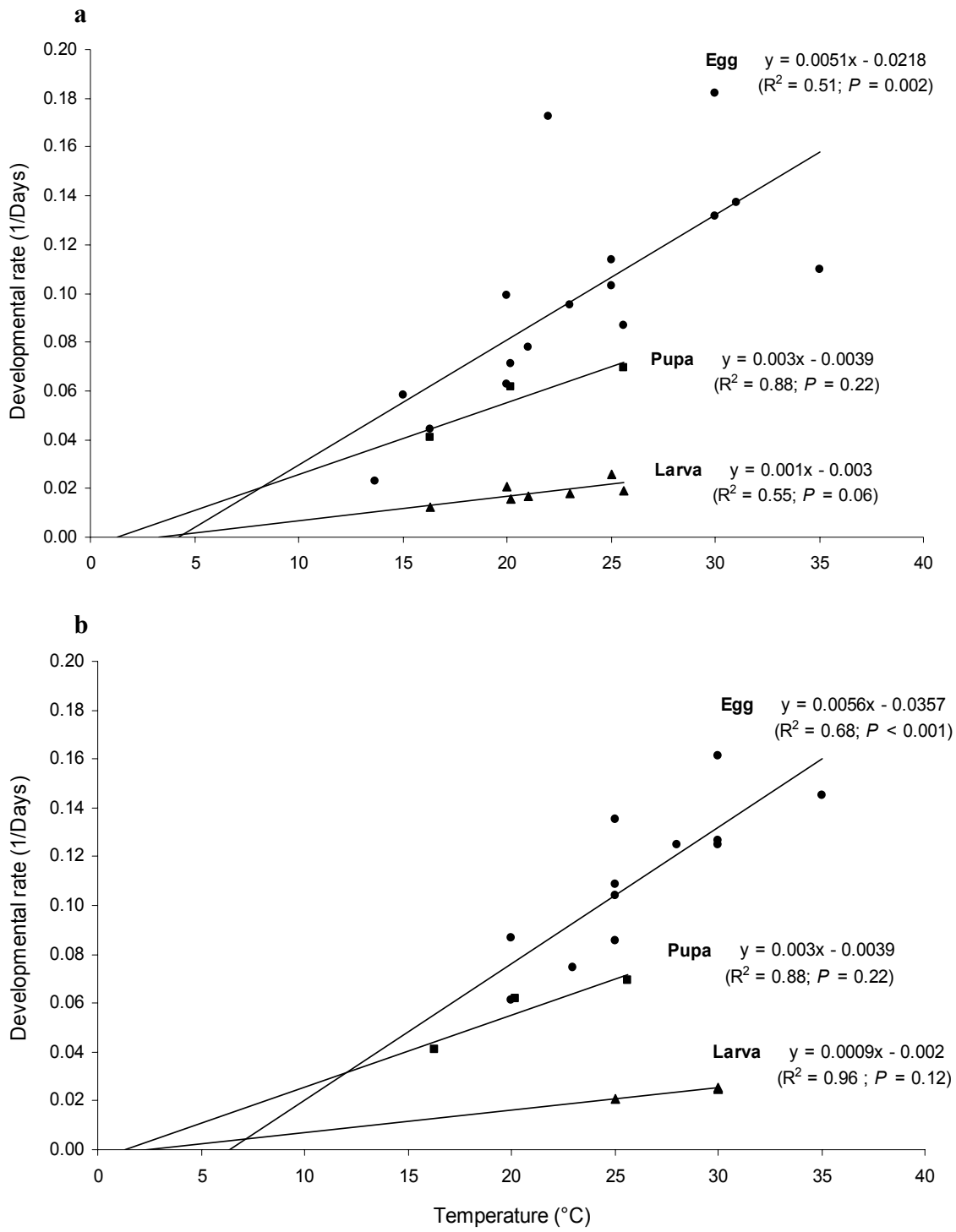


Fig. 2.1. Temperature dependent development of (a) *Neochetina bruchi* and (b) *Neochetina eichhorniae* egg, larval and pupal stages using the linear intercept method (Campbell *et al*. 1974).

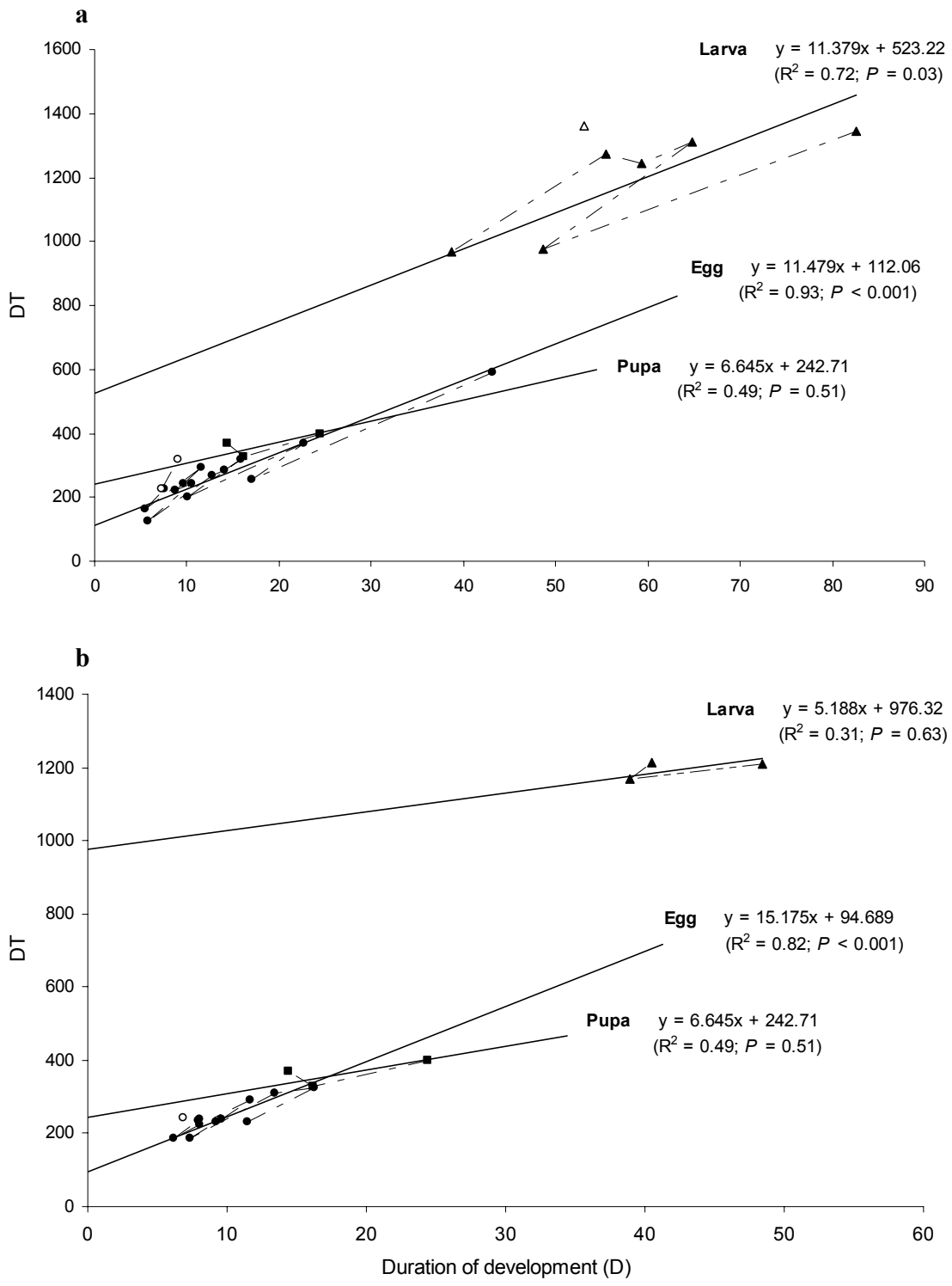


Fig. 2.2. Temperature dependent development of (a) *Neochetina bruchi* and (b) *Neochetina eichhorniae* egg, larval and pupal stages using the reduced major axis regression technique (Ikemoto and Takai, 2000). Unshaded points were omitted from the regression.

Table 2. 3. Lower developmental thresholds (*t*) and thermal constants (K) for immature stages of *Neochetina bruchi* and *Neochetina eichhorniae* using two line fitting methods. Data compiled from Abjar and Bashir (1984); Coetzee, unpub.; Chikwenhere (2000); DeLoach and Cordo (1976a), Stark and Goyer (1983), Shih *et al*. (1994), Wilson (2002).

1. LINEAR INTERCEPT:

Species	**n**	***t* ± SE (°C)**	**K ± SE (°D)**
Neochetina bruchi			
Egg	16	4.2 ± 0.9	194.4 ± 50.7
Larva	7	3.1 ± 0.9	1015.5 ± 413.5
Pupa	3	1.3 ± 0.9	337.9 ± 123.8
All	-	2.9	1547.8
Neochetina eichhorniae			
Egg	12	6.4 ± 0.9	178.7 ± 38.7
Larva	3	2.2 ± 0.9	1101.8 ± 213.7
Pupa *	3	1.3 ± 0.9	337.9 ± 123.8
All	-	3.3	1618.5

2. REDUCED MAJOR AXIS REGRESSION:

Species	**n**	***t* ± SE (°C)**	**K ± SE (°D)**
Neochetina bruchi			
Egg	14	11.5 ± 0.9	112.1 ± 14.5
Larva	6	11.4 ± 3.0	523.2 ± 179.3
Pupa	3	6.7 ± 4.7	242.7 ± 89.3
All	-	9.8	878.0
Neochetina eichhorniae			
Egg	11	15.2 ± 2.2	94.7 ± 22.2
Larva	3	5.2 ± 4.3	976.3 ± 184.9
Pupa *	3	6.7 ± 4.7	242.7 ± 89.3
All	-	9.0	1313.7

* Data substituted from *Neochetina bruchi*.

returned more realistic values for *t*, 9.8°C and 9.0°C for *N. bruchi* and *N. eichhorniae* respectively (Table 2.3).

Due to the paucity of larval and pupal developmental data used for the linear intercept regressions, the optimum temperature range or linear proportion of the relationship between temperature and rate of development could not be determined with any statistical confidence for either weevil species. Therefore, data points that may lie outside this

temperature range might have been included in the linear array resulting in unreliable estimates of *t* and K. Fitting the straight line x = D on y = DT to the same data allowed data points that deviated significantly from the linear tendency to be removed from the final regression. For *N. bruchi* egg development, two points above 31°C were omitted from the final regression indicating an optimum temperature range of 13 to 30°C. Larval development had one point at 26°C omitted limiting the optimum temperature range to between 16 and 25°C. However, due to a lack of data points below 13 and 16°C, it is uncertain whether this range could extend beyond these lower limits. No points were removed from the pupal development regression due to limited data. For *N. eichhorniae* egg development, one point at 35°C was omitted indicating an optimum temperature range between 20 and 30°C although this range undoubtedly extends below this lower limit. No points were removed from the larval development regression due to the low number of data points.

No data for *N. eichhorniae* pupal development exist so data for *N. bruchi* were substituted for this purpose. The reduced major axis regression technique allowed for easier estimation of the temperature range which best approximates the linear section of the development curve. This provided better fitting of the regression line, higher R^2 values, and thus more reliable parameter estimates. Combining developmental data for both weevil species provided a more robust and inclusive dataset by improving the regression fits relative to those produced for *N. eichhorniae* alone. As before, although the regression fits were better for *N. eichhorniae* (Fig. 2.3a), the linear intercept method returned an unrealistically low developmental threshold and long duration of development. The reduced major axis regression method (Fig. 2.3b) provided both suitable fits and good estimates for both *t* and K (Table 2.4).

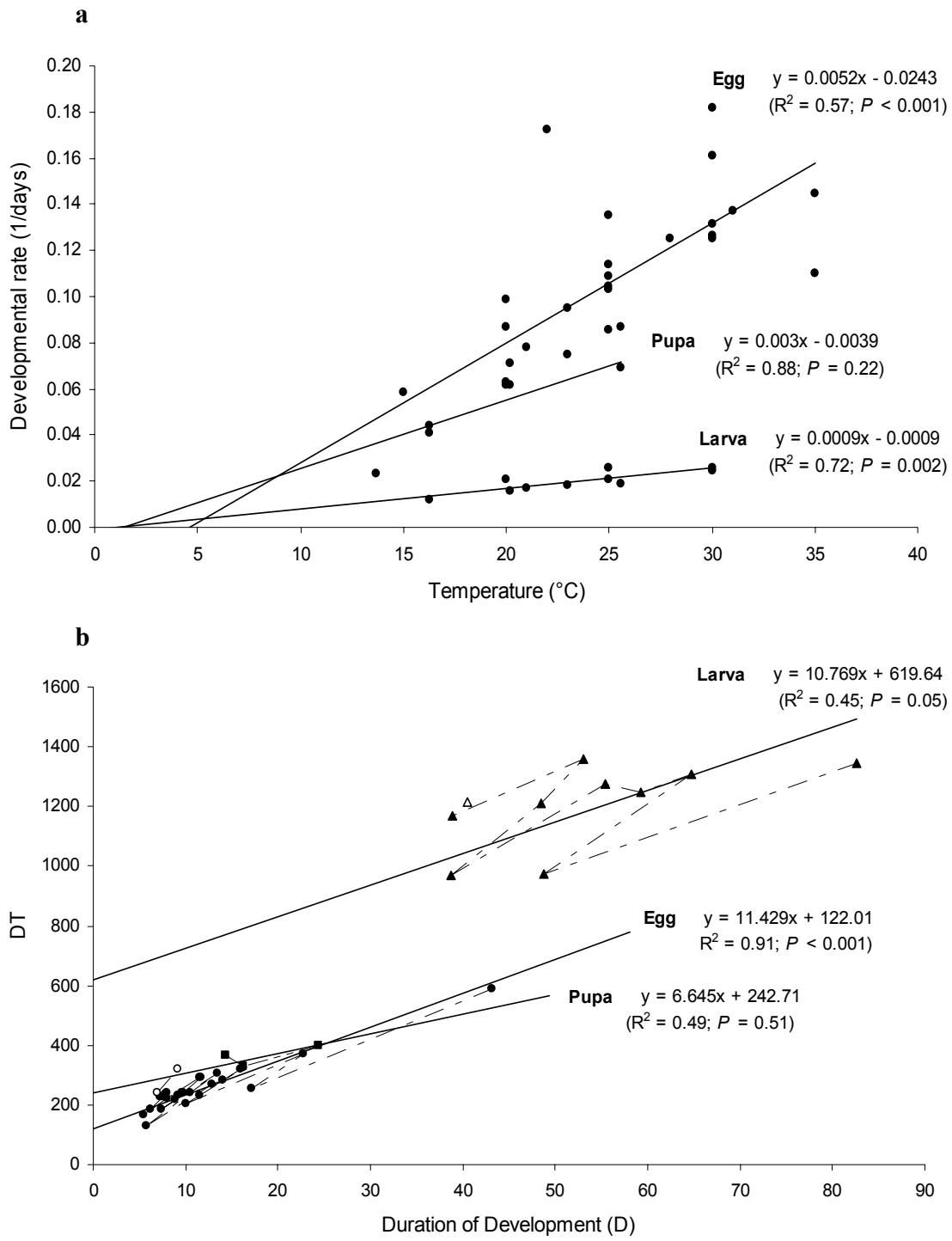


Fig. 2.3. Temperature dependent development of *Neochetina bruchi* and *Neochetina eichhorniae* combined using (a) the linear intercept (Campbell *et al*. 1974) and (b) reduced major axis regression (Ikemoto and Takai, 2000) methods. Unshaded points were omitted from the regression.

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

	LINEAR INTERCEPT		REDUCED MAJOR AXIS REGRESSION	
Stage	***t* ± SE (°C)**	**K ± SE (°D)**	***t* ± SE (°C)**	**K ± SE (°D)**
Egg	4.7 ± 0.8	191.9 ± 32.8	11.4 ± 0.7	122.0 ± 10.1
Larva	1.0 ± 0.9	1135.7 ± 248.9	10.8 ± 3.0	619.6 ± 168.7
Pupa	1.3 ± 0.9	337.9 ± 123.8	6.7 ± 4.7	242.7 ± 89.3
All	2.3	1665.6	9.6	984.4

2.3.2 Assessment of the effects of low temperature and frost on water hyacinth

Distinct frost events were observed and easily distinguishable at each of the sites. Temperatures declined over the first 10 to 11 weeks at all sites and remained consistently low for the remainder of the experiment (Figs 2.4-6). Water temperature correlated best with plant growth trends whilst canopy temperature allowed the dates of individual frost events to be determined. Air temperature followed the same diurnal pattern exhibited by the canopy profile but with greater variability and less consistency in terms of daily maximum and minimum temperatures, and as such was omitted from analyses. Of the three monitored sites, the lower dam at Delta Park was found to be significantly colder ($F_{2, 375} = 35.38$, $P < 0.001$) with a mean daily water temperature of 10.1 ±0.3°C and extremes of water, canopy and air temperature dropping as low as 5.5°C, -4.3°C and -3.0°C respectively. Mean daily water temperature at the upper dam (12.4 ±0.3°C) and at the pool on the university campus (13.3 ±0.2°C) were found to be not significantly different from each other. The upper dam was the least extreme site with water, canopy and air temperatures only dropping as low as 7.0°C, 0.5°C and 2.5°C respectively. Water temperature in the campus pool dropped to 6.5°C, with canopy and air temperature dropping as low as 0°C and 1°C respectively. These profiles fluctuated more extensively than at the other two sites due to the relatively shallow water depth, translating into a higher mean water temperature, making the campus pool the warmest of the three sites.

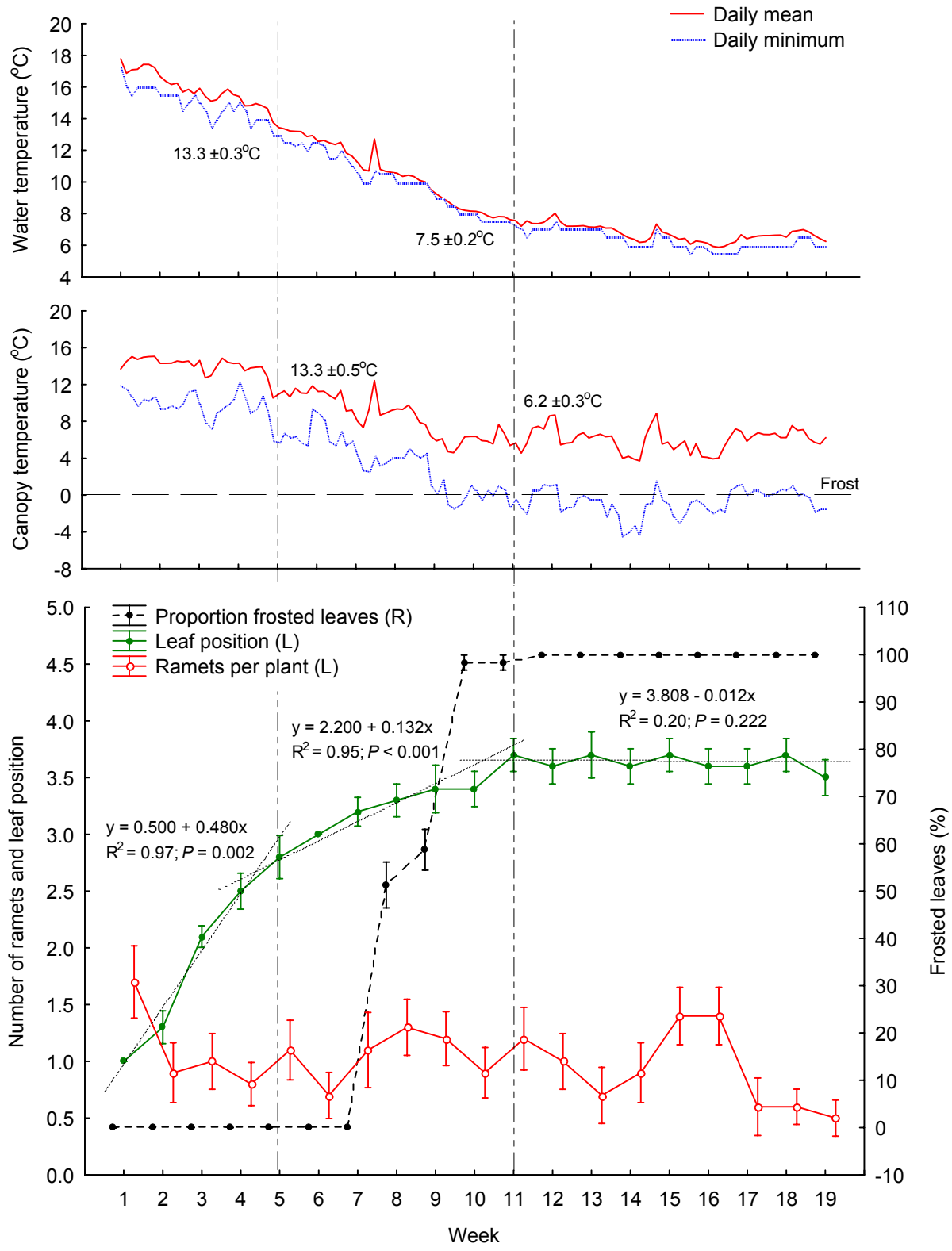


Fig. 2.4. Water hyacinth growth and ramet production (mean of 10 plants ±SE) at Delta Park lower dam, in response to frost and daily mean and minimum water and plant canopy temperature from March to July 2006. Vertical lines separate distinct rates of leaf production.

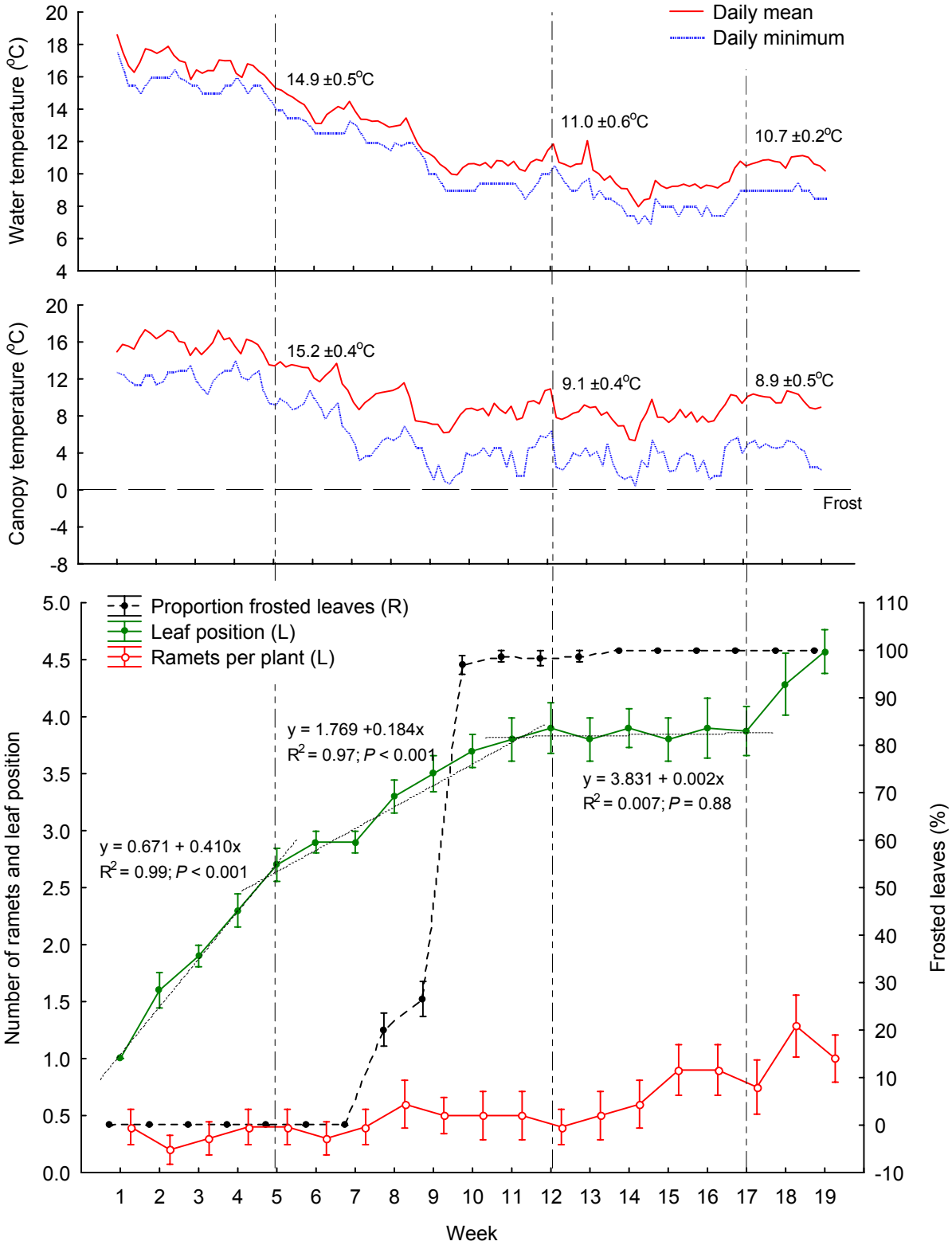


Fig. 2.5. Water hyacinth growth and ramet production (mean of 10 plants ±SE) at Delta Park upper dam, in response to frost and daily mean and minimum water and plant canopy temperature from March to July 2006. Vertical lines demarcate distinct rates of leaf production.

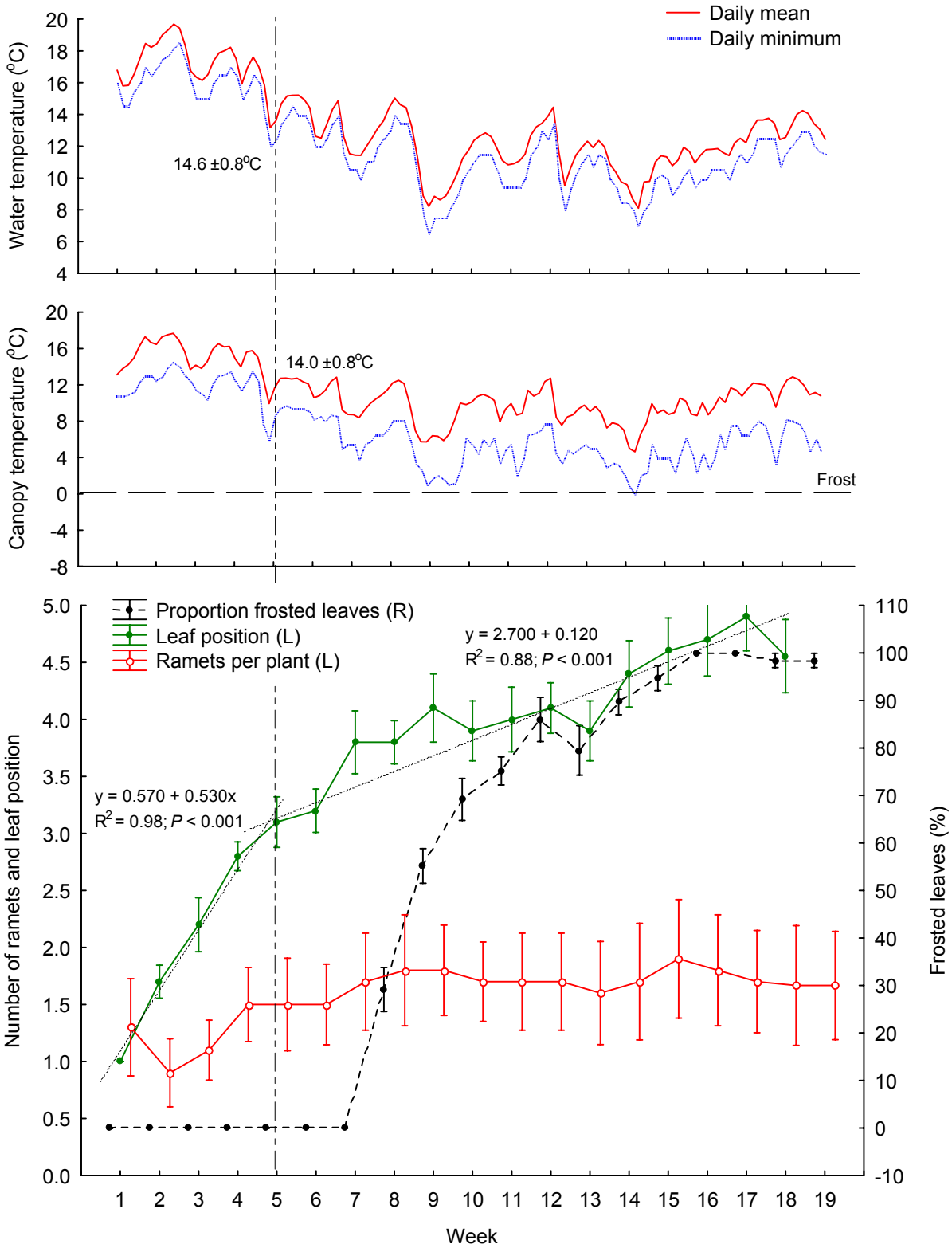


Fig. 2.6. Water hyacinth growth and ramet production (mean of 10 plants ±SE) on a 4.9 m^2 plastic lined wire mesh pool at the University of the Witwatersrand campus in response to frost and daily mean and minimum water and plant canopy temperature from March to July 2006. Vertical lines demarcate distinct rates of leaf production.

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

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

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

Water hyacinth growth was more strongly correlated to water temperature ($r = 0.68$) than with canopy temperature ($r = 0.24$). A positive correlation was found between the rates of leaf production from all sites with increasing water temperature (Fig. 2.7). Assuming a

linear relationship, allowed for the fitting of the standard curve (y = -0.2836 + 0.0411x) to describe leaf production in response to water temperature. This line however indicates a threshold temperature for plant growth of 6.9°C, 3°C lower than expected when compared with the literature (Gopal, 1987).

Conditions at the campus pool produced consistently high numbers of ramets per plant relative to the other sites. Ramet production continued throughout winter at all sites, picking up slightly after week 13 in conjunction with slow rises of mean canopy and water temperature (Figs 2.4-6). The numbers of ramets produced per plant did not correlate significantly with water or canopy temperature at any site. However, fluctuations in the number of ramets per plant did roughly follow the canopy temperature profile at the bottom dam. A weekly mean of the daily maximum temperatures provided

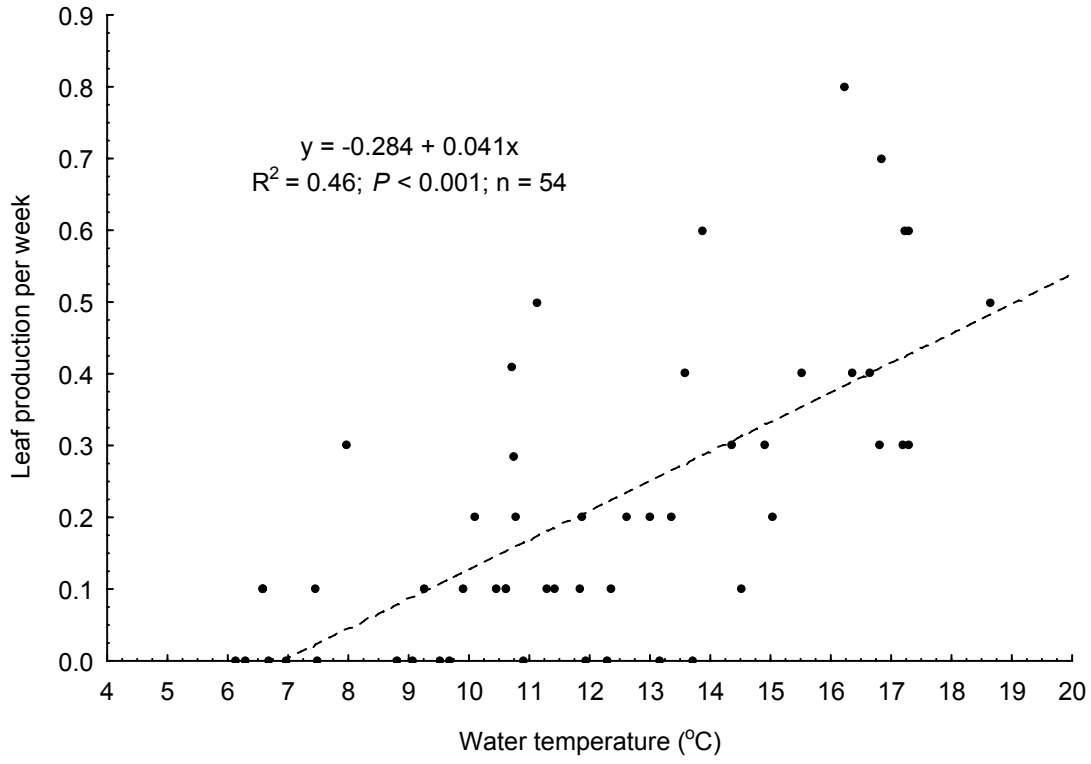


Fig. 2.7. Rate of water hyacinth leaf production per week in response to water temperature. Compiled from three highveld sites between March and July 2006.

the best correlation ($R^2 = 0.11$; $P = 0.17$; r = -0.34) with ramet production and although not a significant fit, went some way to explaining this variation. A decrease in daily maximum temperature therefore coincided with a decrease in the number of ramets produced, however, although only indirectly measured, frost-induced ramet mortality, and reduced plant density and consequent increased exposure of plant crowns to light, appeared to have a stronger influence on ramet production.

Frosted leaves provided some protection for developing ramets. This was evident at the upper dam where significantly longer petiole lengths ($F_{2, 237} = 39.16$, $P < 0.001$) up to the time of first frost allowed for a more sustained and consistent rise in the number of new ramets towards the end of winter. By comparison however, the total number of ramets produced per plant at the upper dam was significantly lower ($F_{2, 507} = 50.30$, $P < 0.001$) than that seen at the other two sites. More ramets were produced at the campus pool, most likely facilitated in part, by higher mean temperatures, as well as lower plant densities, although this was not significantly higher than at the lower dam despite the more severe frost.

Frost damaged leaves were easily identified and were first recorded after week seven at all sites. Canopy temperatures dropped as low as -3°C with daily lows of less than 0°C for every night between weeks seven and eight at the lower dam. Visual inspections after this initial cold spell showed half of the laminas per plant to be frost damaged, the majority of which had between 50 and 100% of the lamina area damaged. Canopy temperatures were slightly higher between weeks eight and nine, only dropping as low as 0°C on three consecutive nights. This resulted in only a small increase of frost damaged leaves. By the following week however, with only two daily lows of under 0°C between weeks nine and 10, 98% of laminas were damaged by frost and of those affected, between 75 and 100% of the lamina area had browned and died. Canopy temperatures were not as extreme at the upper dam as those recorded at the lower dam. As plants were not sheltered, for example, under overhanging vegetation, this was most likely a consequence of the park's topography or the temperature recording device being slightly buffered from extremes due to the longer petioles at the site. Nevertheless, frost damage was observed

following daily lows of 3°C on three consecutive nights during week seven, but caused only limited, less than 5% of the lamina area, to be affected. By week nine, frost damage was only evident on 30% of the observed laminas but by week 10 this figure jumped to 97%. Between weeks nine and 10, six consecutive nights with lows of less than 3°C resulted in between 75 and 100% of the lamina areas of almost all the leaves observed being killed. Frost damage was far less abrupt at the campus pool with daily canopy lows of between 1 and 7°C from week seven to week 10 resulting in 25 to 75% of the lamina areas of 70% of observed leaves being damaged. Frost damage was evident on 100% of leaves observed by week 16 by which time between 50 and 100% of the affected lamina areas were dead. From these data, it is evident that frost is capable of significantly changing plant architecture in a very short space of time which will certainly have implications for resident natural enemies.

2.3.3 Evaluation of insect performance and plant productivity

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

Weevil performance: Fluctuating temperature

Distinct differences were found between the treatment and control chambers in terms of weevil feeding, reproduction and survivability. No eggs were found on any sampled plants in the treatment chambers of either weevil species for the duration of the experiment. By contrast, one egg was found at week 5 in the *N. bruchi* control chamber and another two at week seven. More eggs were found on plants of the *N. eichhorniae* control chamber with four recorded at week six.

Higher levels of weevil mortality were evident at the colder temperatures (Fig. 2.8). For *N. bruchi*, significantly less weevils survived ($\chi^2 = 20.66$; d.f. = 1; $P < 0.001$) for the full duration of the experiment in the treatment chamber. Mortality in this chamber reached 70% whereas in the warmer control chamber only 41% of the original 120 individuals had died by the end of the eight week period. Mortality was higher for *N. eichhorniae*, (86%) in the treatment chamber ($\chi^2 = 35.36$; d.f. = 1; $P < 0.001$) and fell by only 50% in the control chamber.

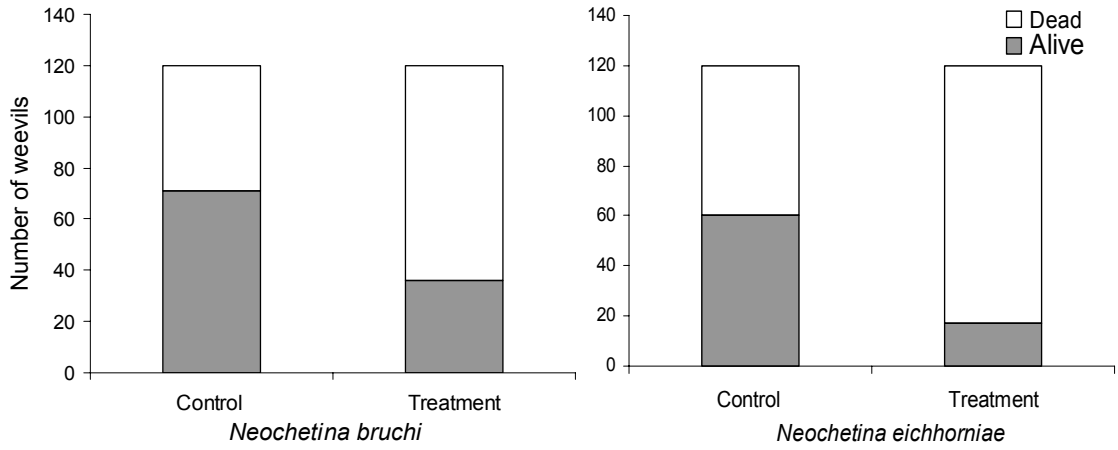


Fig. 2.8. Relative mortality of *Neochetina bruchi* and *Neochetina eichhorniae* in response to simulated low and moderate winter temperature regimes (see Table 2.2) from two sites where the weevils have failed to establish (Treatment) and have successfully established (Control) respectively.

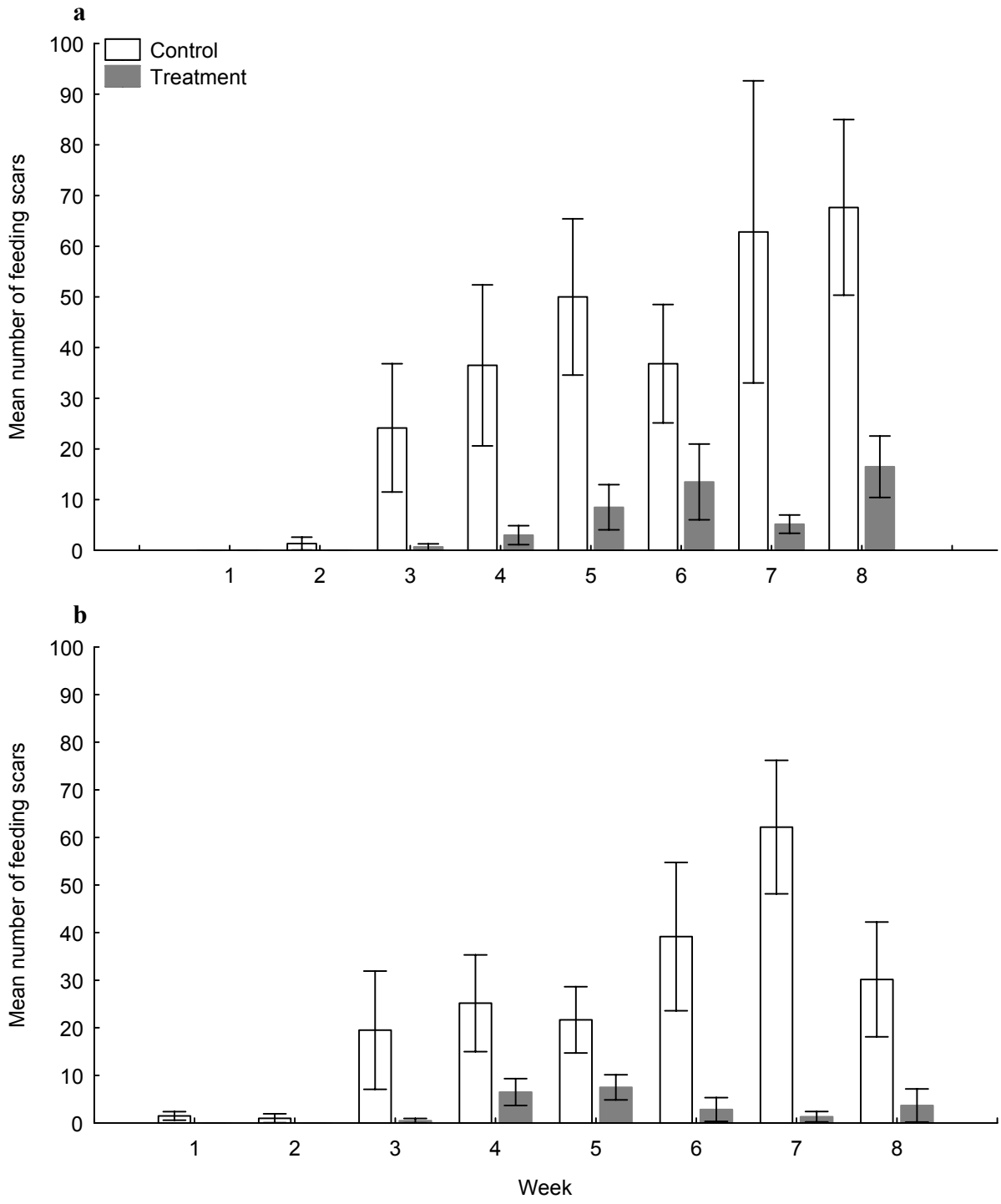


Fig. 2.9. The number of adult feeding scars (mean of the three youngest leaves per plant ±SE) in response to simulated low and moderate winter temperature regimes (see Table 2.2) from two sites where the weevils have failed to establish (Treatment) and have successfully established (Control), respectively for (a) *Neochetina bruchi* and (b) *Neochetina eichhorniae*.

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

Weevil performance: Constant temperature

The above feeding discrepancy in response to differing temperature regimes was confirmed by constant temperature experimentation done in Petri dishes. The rate of feeding scar accumulation was significantly higher ($F_{3, 178} = 47.76$; $P < 0.001$) within chambers maintained at higher constant temperatures. Negligible feeding was recorded at 5°C (0.0063 ±0.0063 scars per weevil per day) relative to that recorded at 12°C which, although not significant, accumulated more (1.79 ±0.33) scars per weevil per day (Fig. 2.10). Significantly more feeding scars per weevil per day were recorded at 15°C (5.10 ±0.56) and again at 20°C (8.42 ±0.85). A strong linear relationship ($r = 0.64$) was found between the accumulation of adult feeding scars and the experimental temperatures used which allowed for the fitting of the standard curve $y = -3.594 + 0.570x$. From the line equation it was estimated that adult *N. eichhorniae* feeding would cease below 6.3°C (*x*-intercept: Fig. 2.10).

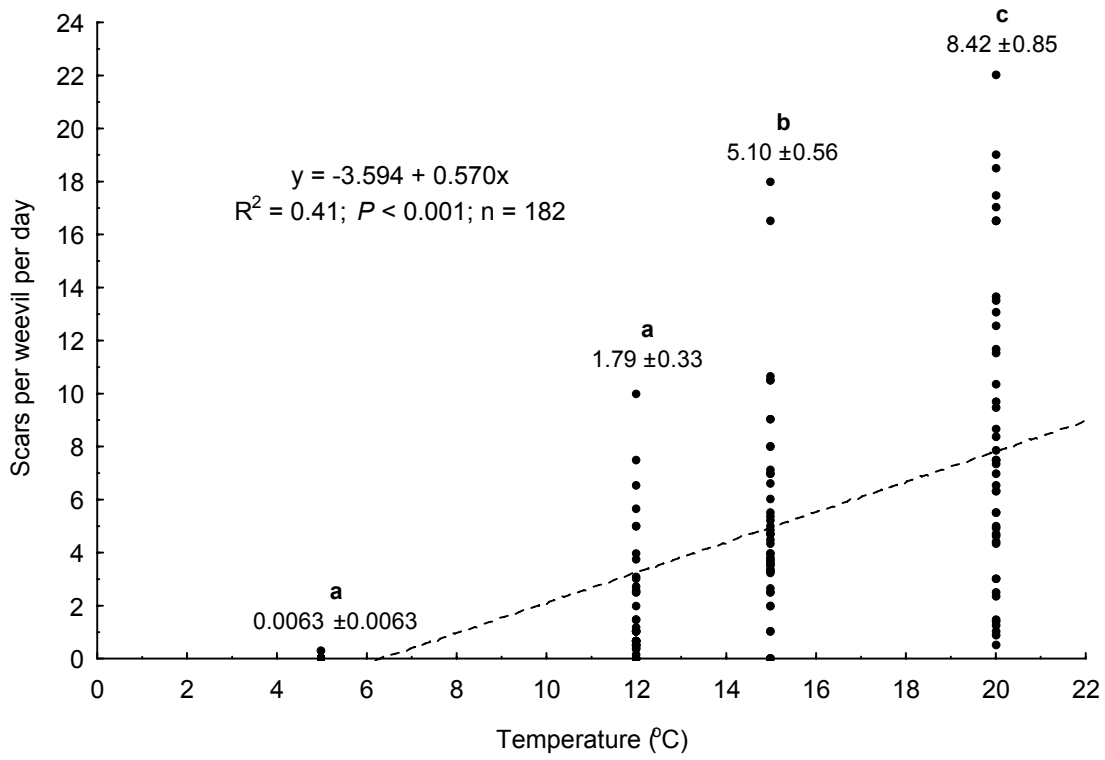


Fig. 2.10. The number of feeding scars produced per adult *Neochetina eichhorniae* weevil per day at four constant temperatures. Weevils were maintained in Petri dishes on cut plant material. Means (±SE) with the same letters are not significantly different at $P < 0.05$ (ANOVA, Tukey HSD test).

Mirid performance

Distinct differences were found in the levels of feeding damage recorded at each temperature regime ($Z = 6.08$; $P < 0.001$) (Fig. 2.11). In the treatment chamber the extent of lamina area damaged by mirid feeding was skewed towards the lower categories with 59% of the observed leaves having no, or less than 5%, of their respective areas damaged. By contrast, leaves in the control chamber were far more damaged by feeding with 44% of the observed leaves having between 50 and 100% of their respective areas affected. These higher levels of feeding damage were undoubtedly a consequence of the larger nymphal population on the plants in the control chamber. No nymphs were produced at air temperatures ranging between 3.2 and 18.4°C around a mean of 10.3°C in the treatment chamber for the duration of the experiment.

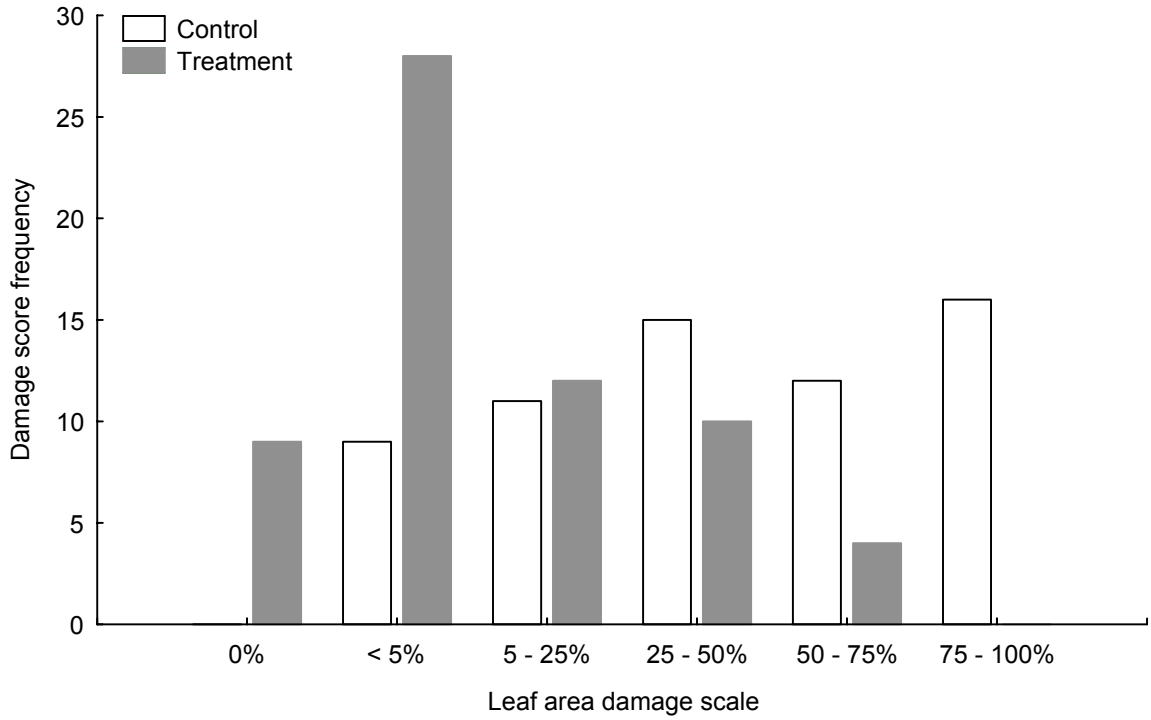


Fig. 2.11. *Eccritotarsus catarinensis* feeding damage scores over an eight week period in response to simulated low and moderate winter temperature regimes (see Table 2.2) from two sites where the mirid has failed to establish (Treatment) and has successfully established (Control) respectively.

By contrast, a daily air temperature fluctuation of between 11.0 and 22.5°C around a mean of 16.8°C in the control chamber, produced small numbers of nymphs by week 5 (Fig. 2.12). At these temperatures, eggs presumably laid at week one took as much as 4 weeks to hatch after which the population of nymphs grew roughly exponentially until the experiment was terminated. Hill *et al*. (1999) reported the egg stage duration for *E. catarinensis* at roughly 9 days at temperatures between 22 and 30°C. Adult population numbers declined steadily in both of the different temperature regimes over the duration of the experiment. However, significantly more adult mirids were recorded per week from the control chamber ($F_{1, 28} = 11.93$; $P < 0.001$) indicating that adult mortality was significantly higher at the colder temperatures.

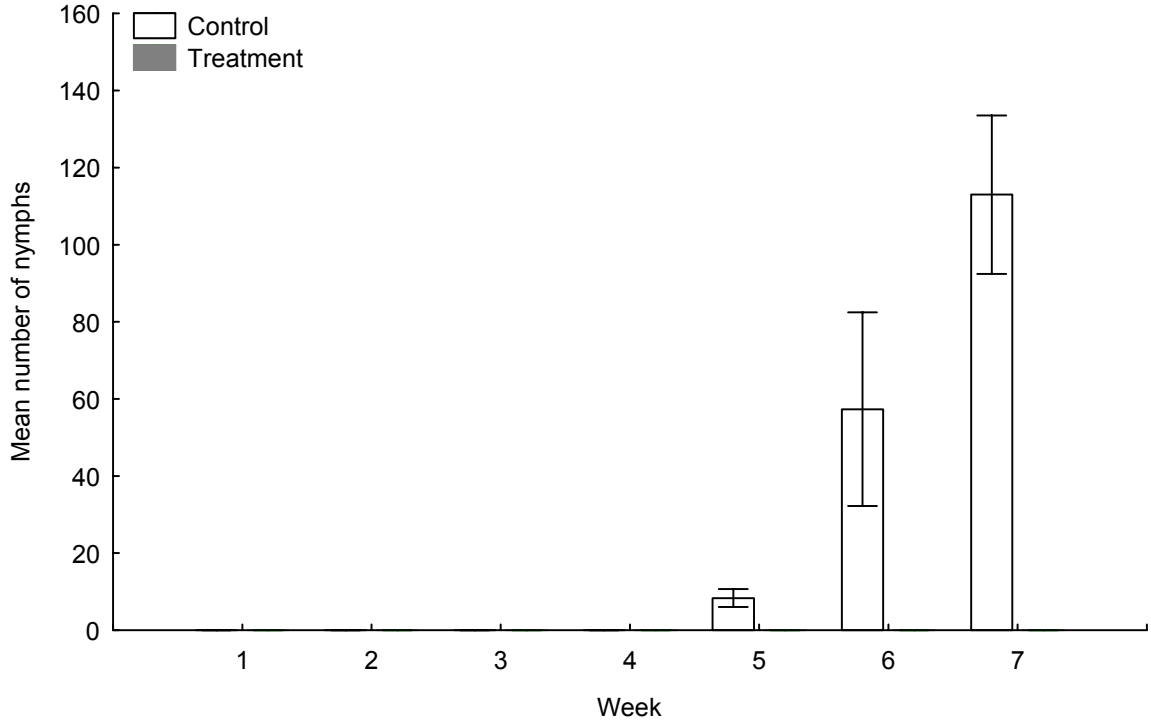


Fig. 2.12. *Eccritotarsus catarinensis* weekly reproduction (mean of three plants ±SE) for a seven week period in response to simulated low and moderate winter temperature regimes (see Table 2.2) from two sites where the mirid has failed to establish (Treatment) and has successfully established (Control) respectively.

Plant productivity

Plant growth and quality during the chamber experimentation differed between the two test temperature regimes in both the weevil and mirid experiments. During the mirid trial, significantly more ramets ($F_{1, 28} = 16.00$; $P < 0.001$) and leaves ($F_{1, 28} = 40.50$; $P < 0.001$) were produced per plant in the control chamber despite significantly higher levels of mirid feeding damage. This trend was mirrored during the weevil trial although it was slightly attenuated by very low temperatures during the first two weeks of the experiment. Problems with the treatment environmental chamber for this initial period caused water temperatures to drop as low as 3.8°C with a daily mean and maximum of 6.0°C and 8.8°C respectively. Canopy temperatures fluctuated around a mean of 6.4°C, with a daily range of between 1.4 and 13.6°C. Canopy extremes were slightly buffered relative to air temperatures which ranged between 0.6 and 17.2°C around a daily mean of

6.7°C. These lower temperatures were analogous to minor frosting resulting in the browning of between 0 and 10% of leaf areas but did not cause any significant rise in leaf senescence or any plant mortality. Nevertheless, for this reason only the plant parameters from the mirid trial were analysed further. Both the number of leaves per plant ($F_{1, 48}$ = 55.34; $P < 0.001$) (Fig. 2.13), as well as the total number of ramets produced ($F_{1, 48}$ = 8.44; $P = 0.01$), was significantly lower in the treatment chamber during the mirid trial. Plant quality within the treatment chamber declined steadily throughout the experiment leading to hardening of lamina and petiole tissue, a decrease in effective leaf area due to browning of the lamina margins, and 40% mortality of plants not sampled. No plant mortality was recorded in the control chamber and plants remained healthy and actively growing throughout the experiment.

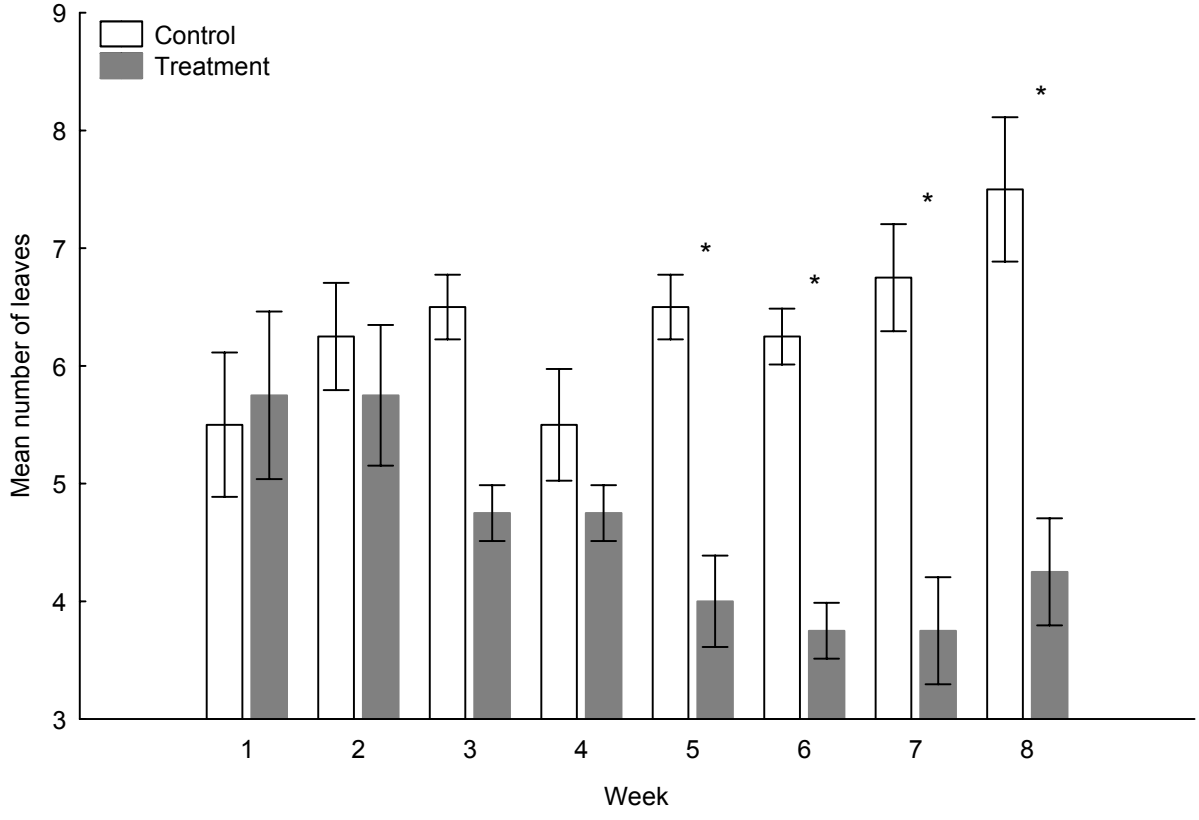


Fig. 2.13. Leaves per plant (mean of three plants ±SE) in response to an eight week exposure to simulated low and moderate winter temperature regimes (see Table 2.2) from two sites where *E. catarinensis* has failed to establish (Treatment) and has successfully established (Control) respectively. Weeks with stars denote significant differences between the treatment and the control at $P < 0.05$ (ANOVA, Tukey HSD test).

2.4 Discussion

2.4.1 Acute temperature effects

This chapter investigated the thermal physiologies of water hyacinth and three of its natural enemies as well as their responses to low, ecologically relevant temperature regimes. The results show that temperature significantly influences insect performance, defined in terms of feeding rates, mortality and reproduction, and indicates that their efficacy as control agents will be limited at lower average temperatures. Water hyacinth shared a similar relationship in that growth rate and plant quality diminished with decreasing temperature, but was less affected in terms of mortality and recruitment. The relative consequences at a population level were therefore less severe for the weed when compared to the insects, suggesting a greater thermal tolerance. This mismatch of tolerances increases the likelihood of a lack of synchrony between water hyacinth and its natural enemies in areas where temperature is limiting.

Goolsby *et al*. (2005) stress that biological control needs to make the transition from a purely empirical method to a predictive science. Predictions of natural enemy efficacy prior to screening and release into a new country would validate the initial investment in the agent, or if predicted not to establish or be effective, could save an investor both time and money. With increasing emphasis placed on integrated approaches to managing invasive plants, predicting an already established agent's efficacy with reference to its locality and seasonality would be invaluable for the timing of other management techniques.

The natural enemies on water hyacinth are severely hampered by low temperatures, both in terms of their persistence and efficacy, especially in regions that are heavily frosted in winter (Hill and Olckers, 2001; Coetzee *et al*. 2007b). Taking the insect's thermal physiology into account, summer conditions throughout South Africa will not be limiting. For this reason, it is paramount to determine how winter affects the insects directly and possibly more importantly, how well they are able to recover from these adverse conditions relative to water hyacinth. Differing tolerances towards temperature could then lead to asynchrony of the plant and insect population structure and growth rates emerging

from winter. Mistimed insect feeding or prolonged population build up following winter would severely limit the level of control achieved, the effects of which could last well into the more favourable summer months. In order to test these assumptions, theoretical models describing water hyacinth, *Neochetina*, and *E. catarinensis* population growth emerging from adverse winter conditions and based on accumulating degree-days were constructed utilising the above reported experimentally derived thresholds.

2.4.2 Phenological model and evaluation of synchrony

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

The assessment of the effects of low temperature and frost demonstrated that water hyacinth growth, at least in terms of leaf production, is largely determined by water temperature. Extrapolation of the line from Fig. 2.7 suggests that leaf production ceases at a water temperature of 6.9°C. However, this temperature threshold seems unrealistically low given that while evaluating plant productivity within a controlled environmental chamber, plants maintained at a mean water temperature of 9.5°C stopped leaf production and experienced increased rates of leaf senescence (Fig. 2.13). This resulted in a steady decrease in the number of leaves per plant in the chamber during the eight week period. At the Delta Park sites leaf production ceased at mean daily water temperatures of 7.5°C at the lower dam and 11.0°C at the upper dam. By week 18 however, new leaves were recorded at the upper dam at water temperatures around a

mean of 10.7°C. Plant growth can therefore be assumed to cease at water temperatures between 9.5 and 10.7°C, which is consistent with Gopal (1987)'s prediction of a developmental zero for water hyacinth of 10°C. This is further justified by the fact that plant growth did not cease at the campus pool with mean daily water temperatures never dropping below 11.5°C (Fig. 2.6). Similarly, while evaluating plant productivity, plants maintained at a water temperature of 14°C remained actively growing for the duration of the experiment. Therefore, plants at the Delta Park sites must be assumed to remain dormant throughout winter until a biofix point or onset of more favourable conditions in the form of rising mean water temperature is reached.

Following the assessment of the effects of low temperature and frost, water and canopy temperature at the lower dam was continually monitored through spring and into summer providing a basis against which plant and insect recovery after winter could be modelled (Figs 2.14 and 2.15). Biofix points for these models were determined as the final day of the first consecutive seven days with a mean temperature above the species relevant developmental threshold occurring after the coldest week in winter. For water hyacinth, daily leaf production was modelled using the line equation $y = -0.2836 + 0.0411x$ derived in Fig. 2.7, from mean daily water temperatures above a threshold of 10.1°C. From Figs 2.14 and 2.15, plant growth was predicted to commence on the 13th of September, producing new leaves at a rate that rose sharply in conjunction with warming water temperature to a rate of 0.56 new leaves per week by the end of December. This rate could conceivably rise much higher if water nutrient levels were conducive to this. Center (1980) found that leaf production rates during Florida summers in the USA, could reach as high as 0.7 new leaves produced per week under high nutrient conditions.

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

model. The grouping of such closely related species in terms of their thermal tolerance is nevertheless justified. Insects occupying the same or very similar ecological niches can be assumed to share a variety of similar adaptations to temperature (Ikemoto, 2003). Consequently, insect strains or closely related species are expected to have correspondingly similar developmental thresholds (*t*) and durations of development (K). Ikemoto (2003) reports on a number of studies which found that K decreased with increasing *t* among related species mainly at Order level but even at higher taxonomic categories. Ikemoto (2003) also showed that within a number of insect families and genera, at least mathematically, a common *t* and K does in fact exist, and proposed that, biologically, this threshold temperature and duration of development represents a physiological optimum that a group of closely related organisms possess. *Neochetina bruchi* and *N. eichhorniae* are very closely related with similar physiology and behaviour, and occupy almost identical niches. Evidence of this can be seen from the rates of development of each life stage (Figs 2.1 and 2.2).

Combining the development for both *Neochetina* species yielded a developmental threshold of 9.6°C with a thermal constant of 984.4°D which was consistent with estimates made for other tropical weevil species that live on aquatic plants. Mazzei *et al*. (1999) found that the milfoil weevil *Euhrychiopsis lecontei* Dietz (Curculionidae) required 309°D above a threshold temperature of 9.8°C to complete the development of its immature stages. Similarly, McConnachie (2004) calculated that the weevil *Stenopelmus rufinasus* Gullenhal (Curculionidae) released against *Azolla filoculoides* Lamarck (Azollaceae) required 256°D above a threshold temperature of 9.2°C to complete its development. The thermal constant was, nevertheless, higher than anticipated compared with these species. Being tropical in origin, developmental thresholds are expected to be roughly similar to other tropical species, but as *N. bruchi* and *N. eichhorniae* are larger in size, the thermal constants would be correspondingly longer as well (Chown and Nicolson, 2004). Indeed, coupled with the calculated developmental threshold, the duration of development or generational turnover is consistent with what has been observed in the field (Stark and Goyer, 1983; DeLoach and Cordo, 1976a). Nevertheless, some degree of error in the absolute values of *t* and K must

be expected due to the relative inconsistency between the data sources from which they were calculated.

It was evident from the evaluation of weevil performance that lower average temperatures caused high levels of mortality, up to 70% in as little as eight weeks. It must therefore be assumed that numbers of adult weevils able to survive through winter until conditions are more favourable will be exceedingly small. Furthermore, Byrne *et al*. (2010) found that the reproductive state of the weevils changed in response to cooling temperatures. Field collections indicated that weevils entered a 'reproductive diapause' up to two months prior to winter, which lasted until spring. Coupled with high rates of mortality, the contribution of overwintering adults to a recovering spring population through egg production will thus be negligible. Additionally, from the assessment of the effects of frost at the lower dam, plant damage as a result of heavy frosting was both severe and rapid. Between 75 and 100% of the lamina area had browned and died in as little as three weeks. *Neochetina* eggs, normally found at the bases of laminas (DeLoach and Cordo, 1976a; Center, 1987), laid prior to these initial frost events would almost certainly be killed, as well as the majority of both first and second instar larvae occupying the upper reaches of petioles. Due to the progressive browning of petioles from lamina to crown observed during winter, only third instar larvae, normally occupying the crown (DeLoach and Cordo, 1976a) are predicted to successfully overwinter in any meaningful numbers able to contribute to a post winter weevil population.

Setting a biofix point for the weevils in the same way as for water hyacinth, overwintering third instar larvae were assumed to pupate following the first consecutive seven days, after the coldest winter week, with a mean water temperature above the larval developmental threshold of 10.8°C (Table 2.4). Larval development was modelled against water temperature rather than canopy temperature due to the close proximity of plant crown, and thus the larva, to the surface of the water. In this way, weevil recovery was predicted to commence when larvae pupated around the 16th of September at which point mean water temperature was well above the pupal developmental threshold of 6.7°C (Fig. 2.14).

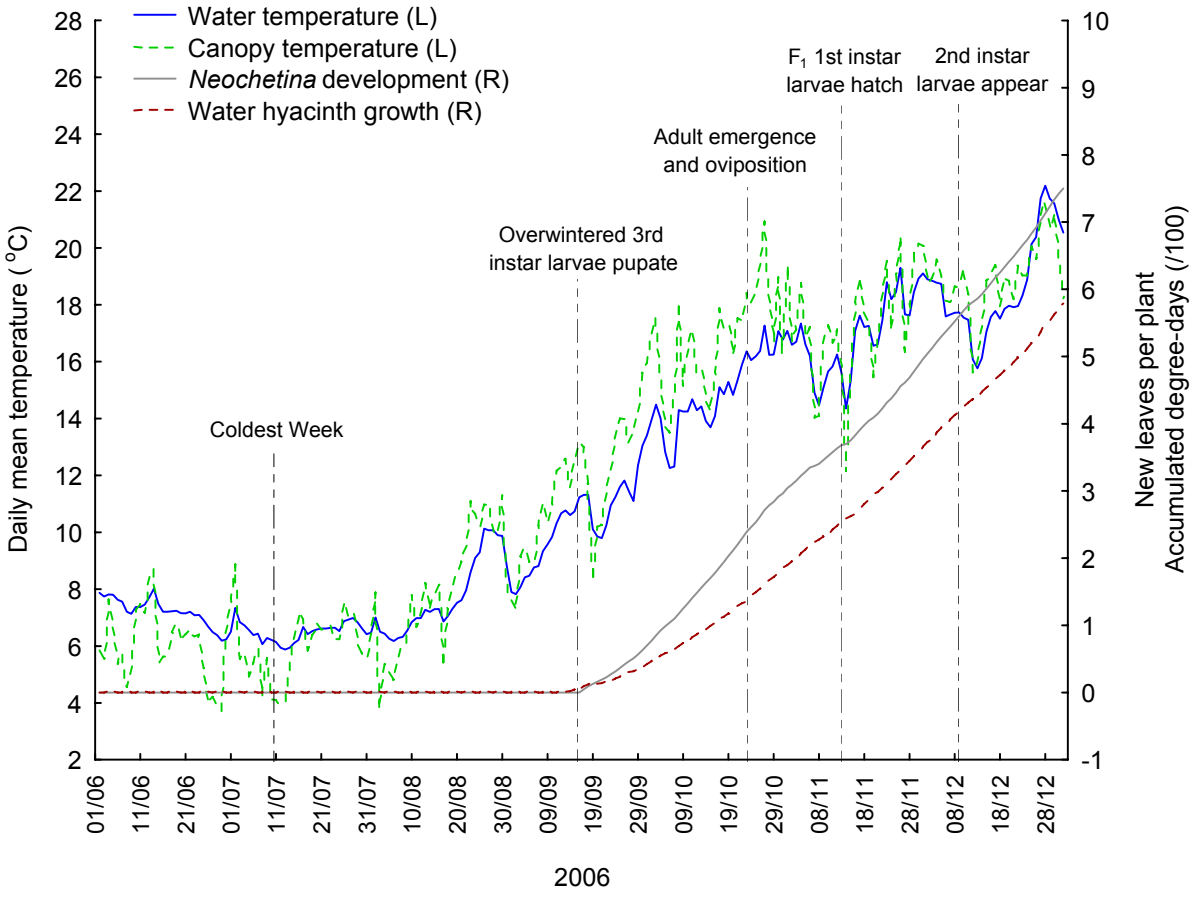


Fig. 2.14. Predicted water hyacinth and *Neochetina* recovery following adverse conditions during a highveld winter. Leaf production rate and *Neochetina* accumulation of degree-days during pupation are in response to water temperature. Subsequent *Neochetina* development is in response to plant canopy temperature.

Degree-hours were calculated from hourly measures of water temperature for pupation, and canopy temperature for egg maturation and larval development, and averaged over 24 hours to determine the number of degree-days accumulating per day. The accumulation of degree-days utilising life-history stage specific developmental thresholds was then used to predict the rest of the weevil's post-winter phenology. Adult eclosion was predicted on the 24th of October, 1st instar larval hatch on the 13th of November, and the transition moult to 2nd instar larvae 26 days later on the 9th of December.

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

This model therefore predicts a lag period of roughly 42 days between the onset of water hyacinth growth and the time at which the plant will be subjected to herbivory. Feeding damage at this point, by the emergent adult overwintered population, will be restricted to the leaves with a consequently negligible impact owing to limited weevil numbers and increasing leaf production rates. Realistically then, as the larval instars are the most damaging stage (DeLoach and Cordo, 1976a), the weevils may only begin to significantly impact the weed population when the first instar F_1 generation hatches around 62 days later. Wilson *et al*. (2006) maintains that the late larval instars are the most damaging with 3rd instar larvae having the highest consumption rates. Accordingly, this lag period could extend into early January of the following year when 3rd instar larvae are predicted to appear. By this stage however, the plant is predicted to have produced up to eight new leaves.

As degree-days accumulated during weevil development at similar rates to leaf production, and the recovery of both weevil and plant populations commenced at roughly the same time after winter, differential responses to temperature cannot be the sole factor promoting this lag period. Rather, it is the susceptibility of each *Neochetina* life stage to mortality brought on by low temperature and the consequences of frost for the plant that is most likely to promote this mismatch between plant and weevil phenology. Even before the onset of winter, declining plant quality brought on as a result of cooling temperature, is expected to adversely affect *Neochetina* populations. Center and Dray (2010) showed that plant quality drove the population growth of both *N. eichhorniae* and *N. bruchi*. Lower nutritional circumstances, in particular leaf nitrogen, brought on by nutrient limitation, resulted in decreased fecundity and reduced population growth rates of the weevils. Decreased nutrient uptake as a result of diminishing plant growth rates due to cooling water temperature, coupled with the hardening of plant tissues, as reported during the chamber experiments, could therefore severely limit *Neochetina* population growth leading into winter. During winter, low temperature hampers oviposition and promotes high overwintering mortality of all *Neochetina* life stages. Additionally, frost-induced leaf senescence will kill both eggs and early larval instars, but does not lead to any significant plant mortality, leaving only a reduced 3rd instar larval population able to overwinter. These factors will severely limit the number of individuals that are able to contribute to a post winter population. Therefore, the need for an early season period for pupation, which is prolonged by slow weevil development due to low temperature, allows the weed population to recover from winter relatively untouched.

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

range of favourable temperature for survival, reproduction and development relative to its prey. The authors concluded that because of the subsequent lower intrinsic rate of increase in relation to its prey, *S. punctillum* is unlikely to provide consistent control of the mite. In contrast to the relatively slow population increase of the weevils (*K* selected), water hyacinth (*r* selected) has a far higher intrinsic rate of increase. *K* selected species are generally more suited to stable environments and due to relatively slow reproductive rates and longer generation times are far slower to recover from adverse conditions and are therefore unable to attain large population sizes in unfavourable and highly seasonal environments. Harris (1991) suggests that control is more likely to be achieved with biological control agents that attack their hosts early in a plants lifecycle. Targeting water hyacinth early in its growth season, during post winter recovery would have a far greater impact given the plant's relatively slow growth rate at the time. For this reason *r* selected natural enemies, better able to cope with more stochastic environments, would have a greater potential to overcome this lag period. Faster reproductive rates and shorter generation times will facilitate rapid population increase, enabling an organism to recover far more quickly from adverse conditions, leading to more feeding damage more quickly. Center (1980) touched on this, observing that the moth *N. albiguttalis*, which behaves more like an *r* strategist, was responsible for a different type of age specific leaf mortality as compared to that initiated by *N. eichhorniae.*

Eccritotarsus catarinensis is an *r* strategist having a short generation time (Coetzee *et al*. 2007b) and fast reproductive rate as demonstrated in its environmental chamber evaluation (Fig. 2.12). Accumulating degree-days for the mirid against the same temperature dataset used in Fig. 2.14, it is evident how this different life-history strategy may contribute to better water hyacinth control. A biofix point was calculated in the same way as for the plant and weevils but a developmental threshold of 10.3°C, and only the canopy temperature profile was used. As mean daily temperature within the water hyacinth canopy picked up far sooner than in the water temperature profile, mirid recovery commenced on the 26th of August, 19 days prior to the onset of plant growth which was dictated by cooler water temperatures (Fig. 2.15). Eggs laid at this point were predicted to hatch after a further 33 days and a full generation completed in as little as 66

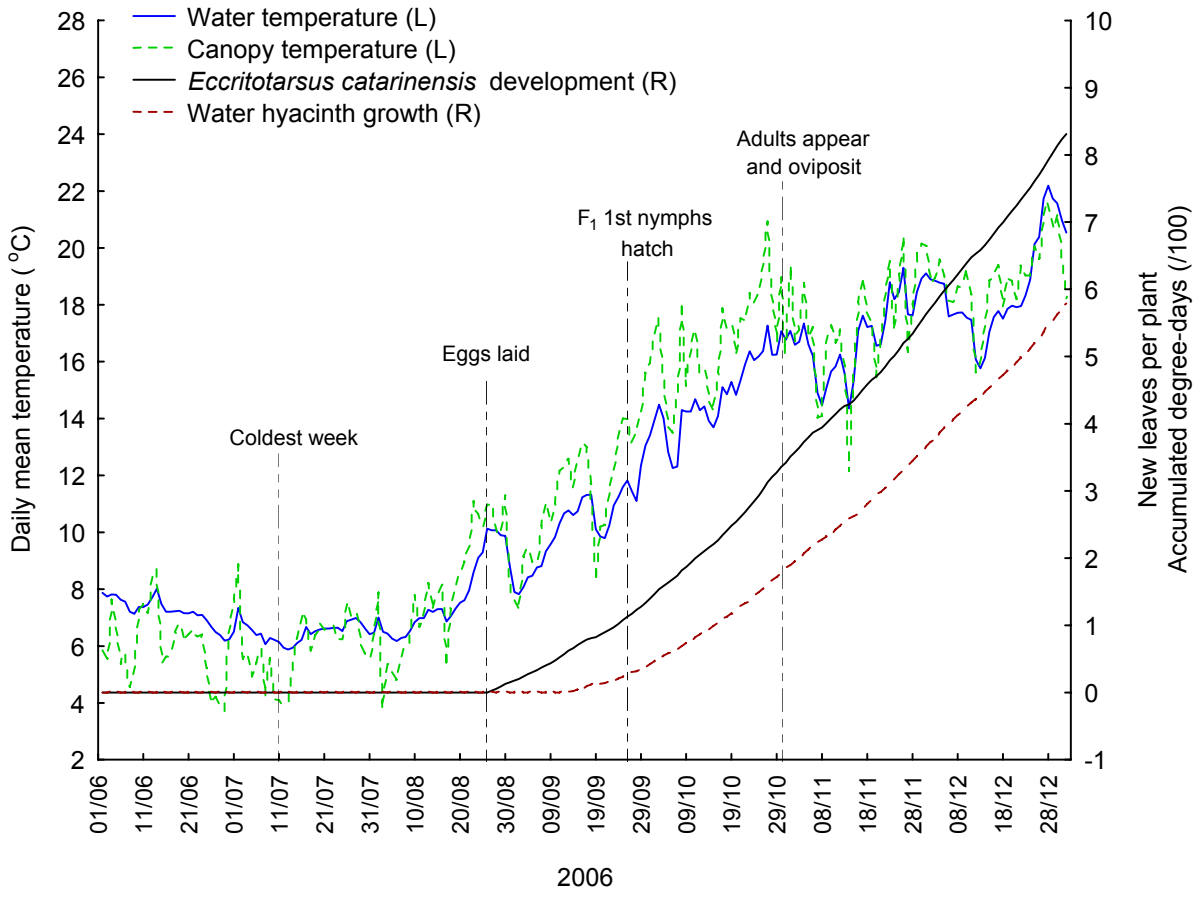


Fig. 2.15. Predicted water hyacinth and *Eccritotarsus catarinensis* recovery following adverse conditions during a highveld winter. Leaf production rates are in response to water temperature. *Eccritotarsus catarinensis* accumulation of degree-days is in response to plant canopy temperature.

days, around the 31st of October. Adult mirids are the most likely life-history stage to successfully overwinter due to their increased probability of finding both food and suitable habitat owing to their high motility relative to the flightless nymphal instars. Consequently, egg and nymphal mortality during winter is presumed to be high and their contribution to a recovering post winter population negligible. Nevertheless, a successfully overwintering mirid population cannot be guaranteed. Although adult mortality was significantly less in the warm control chamber relative to the cooler treatment chamber, high levels of mortality, up to 75%, were observed in both chambers by the end of the experiment. This suggested an adult life span of approximately seven

weeks and is consistent with the estimate by Hill *et al*. (1999) of approximately 50 days, and suggests that a short adult life span, rather than temperature, was responsible for the observed mortality. Additionally, no reproduction was recorded at mean daily air temperatures of 10.3°C in the colder chamber for the duration of the seven week period. Daily canopy temperature at the lower dam, which fluctuated around a mean of 8.4°C for 15 weeks prior to the biofix point, would almost certainly preclude reproduction, and with no recruitment during winter, mirid populations are anticipated to die out each winter due to this short life span. Indeed, repeated attempts to establish the mirid at the lower dam site in Delta Park have failed (Coetzee, 2003).

In this way, these models can be seen as purely theoretical and will constitute a best case scenario for each organism emerging from adverse thermal conditions. A variety of factors that might shorten or prolong different stages of the models are omitted. Initial biofix points, as well as points of transition between growth rates or insect life stages can only be estimated. For instance, Bennet and Zwolfer (1968) speculated that *N. bruchi* has a preoviposition period of 19-20 days. Following eclosion, this model assumes weevils are able to lay eggs immediately and thus do not require a maturation or mating period prior to oviposition. Increased survivorship of adult weevils and early larval instars through winter could also lead to larger population numbers and the inclusion of more damaging life stages sooner than predicted.

In this study, both weevils have largely been considered together, but different proportions of each species at a site could influence the relative impact of this lag period on the plant. Center and Dray (2010) showed that *N. bruchi* and *N. eichhorniae* fecundity differed based on plant quality. *N. bruchi* population growth increased more rapidly than *N. eichhorniae* when feeding on plant tissue with a high nutritional value, whereas *N. eichhorniae* population growth could continue under a wider range of plant nutritional conditions. Therefore, at post-winter sites dominated by *N. bruchi*, damage should appear earlier because populations with increased fecundity relative to *N. eichhorniae*, would be quicker to respond to the new, high quality, spring growth (Center and Dray, 2010). Nevertheless, as differences in egg loads were slight, the authors suggested that the

differing reproductive performances of the two species could be due to density dependent larval survival as described by Wilson *et al*. (2006), and not fecundity alone. In this study, *N. eichhorniae* was able to lay more eggs in colder conditions relative to *N. bruchi* which could contribute to a larger, more damaging post-winter first instar population. This result was, however, not significant and is contrary to DeLoach and Cordo (1976a) who found that *N. bruchi* laid more eggs at all their test temperatures relative to *N. eichhorniae*. The environmental chamber evaluation of weevil performance suggested that *N. bruchi* is better able to tolerate colder conditions in terms of adult survival and feeding. Greater numbers of feeding scars recorded in the warmer chamber could reflect the fact that mortality in the colder chamber was significantly higher translating into fewer feeding individuals. Differences in weekly accumulations of feeding scars only by week seven and eight provided further evidence of this. Nevertheless, from Fig. 2.10 it is evident that feeding rates of *N. eichhorniae* were slower as a consequence of colder temperatures but the environmental chamber evaluation showed that this response did not differ significantly between the weevil species. Therefore, reduced feeding rates coupled with temperature dependent mortality is one of the most likely causes of negligible levels of feeding and consequently diminished levels of control at colder sites.

Frost severity during winter is another compounding variable which will affect the realised impact of insect natural enemies on water hyacinth populations. Whilst water temperature dictates the rate of plant growth, frost damage is most often linked to plant and leaf mortality (Wilson *et al*. 2001). In this way, the severity of frosting has the potential to influence water hyacinth population density and thus, any density dependent processes as well. From the assessment of the affects of frost, one such example appears to be the production of ramets which continued throughout winter at all three sites. Therefore, whilst frost limits natural enemy populations by directly causing mortality or through density dependent regulation by limiting the number of suitable petioles in which larvae can develop, it promotes ramet production and seldom causes plant mortality. Center and Spencer (1981) found that the vegetative regrowth of a water hyacinth population following defoliation by winter freezes in north-central Florida was characterised by three distinct phenological phases. The first was characterised by a

reorganising of the distribution of biomass between the roots and the aerial parts. The second saw an increase in plant density in response to available interplant space, and the third was characterised by an increase in plant size due to competition for light. Many plants respond morphologically to decreases in light, as a result of competition, in order to increase their potential to intercept light. These responses include elongation of stems and petioles, as well as increases in leaf area or resource portioning to leaves (Méthy *et al*. 1990), or through the modification of photosynthetic rates (Méthy and Roy, 1993). In a study of the effects of light quality on the morphology and growth of water hyacinth, Méthy and Roy (1993) found that fewer ramets were produced under reduced levels of far-red radiation. In short, conditions simulating low light availability within a water hyacinth canopy did not stimulate the production of new ramets. Further evidence of this can be seen in the study by Richards (1982), which showed that axillary buds of plants inside a water hyacinth mat rarely produced ramets whereas plants on the mat edge, characterised by shorter inflated petioles, produced numerous ramets. Thus, it can be assumed that increased light availability due to high levels of leaf senescence brought on by severe frosting has the potential to stimulate the production of new ramets.

2.4.3 Conclusion

Although both water hyacinth and its natural enemies are negatively affected by low average temperatures, the relative consequences for each at a population level are quite different. Similar thresholds of development mean that periods available for growth in areas where winters are limiting will be roughly the same for both the plant and insects. However, the ability to overwinter successfully appears to the primary cause for limited control at colder sites. Reduced recruitment as well as high susceptibility to cold- and frost-induced mortality of all life-history stages, push the insect populations through a bottleneck each winter or can even cause local extinctions. Surviving post winter insect populations are therefore small, inflict minimal damage due to reduced feeding rates, are slow to recover in the case of the weevils, and consequently, impact on recovering plants is negligible. Free from early season herbivory, the fact that ramet production continues through winter, and frost most often does not lead to significant levels of plant mortality,

water hyacinth populations are able to recover quickly and outpace the detrimental effects caused by insect feeding well into the growth season.

It is therefore paramount to accurately predict the seasonality of both water hyacinth and its natural enemies to assure maximum success of any augmentative management techniques, for example, herbicide treatments. Undoubtedly, this will be most pivotal in regions where winters are limiting. In these areas, modelling should provide a means of predicting at least the first distinct, and most vital, according to Harris (1991), post winter early season cohorts of both plant and insect populations. Subsequent cohorts or generations are likely to become less prominent and more difficult to predict as the year proceeds and generations overlap. Similarly, in regions with less severe winters and thus less distinct seasonality, things will become less clear. Although providing invaluable predictions, many questions therefore remain unanswered and as such these models will require extensive ground truthing.

The data collected throughout this chapter is applied to a variety of field scenarios in South Africa in chapter 3. The physiological thresholds are used to develop degree-day based models which are able to accurately illustrate the phenologies and generational turnover of both the *Neochetina* weevils and *E. catarinensis* in the field. These models are then validated against datasets collected monthly at each field site. Plant developmental data, such as leaf production rates in response to water temperature, are also used to improve the accuracy and predictive capabilities of these models. In addition to this, plant and insect threshold temperatures calculated in this chapter are used to determine the amount of thermal stress accumulating at each of these field sites. The resulting consequences for biological control of water hyacinth in South Africa are discussed.

CHAPTER 3 – DESCRIBING POPULATION DYNAMICS OF WATER HYACINTH BIOLOGICAL CONTROL AGENTS IN RELATION TO MICROCLIMATE

3.1 Introduction

3.1.1 Degree-day accumulation

Degree-day values are used for modelling because they quantify phenological development (Snyder *et al*. 1999; Cesaraccio *et al*. 2001; Reji and Chander, 2008). Degree-day calculations allow the duration of plant and insect development to be estimated by adding up the number of heat units that occur above the lower temperature threshold in a certain time period (Wagner *et al*. 1984; Pilkington and Hoddle, 2006). This physiological approach enables the theoretical duration of different life stages, the number of generations in a given time period (Pilkington and Hoddle, 2006), and the timing of phenological events to be calculated (Fidanza *et al*. 1996). Due to its relative simplicity in terms of data input and formulation, this method is widely used, yielding approximately correct values which have shown good predictive capability in the field (Wagner *et al*. 1984; Skinner *et al*. 2006).

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

Such problems include the assumption that plant and insect development is always directly or linearly related to temperature (Miller, 1992; Herms, 2004). Bonhomme (2000) maintains that this relationship is not always linear. At a basic level, degree-day models use the assumption that the growth of an organism depends on the rate of various enzymatic reactions. However, the rates of such reactions can be influenced by the availability of water, nutrients and photosynthates for plants; and food and water for animals, and must be present in adequate amounts for optimal development which can only then be predominantly controlled by temperature. Similarly, the availability of enzymes, which are regulated hormonally, can also lead to reduced or unpredictable growth rates (Higley *et al*. 1986).

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

Another confounding variable is the assumption that poikilothermic organisms cannot regulate their body temperature. Among insects especially, species use both behavioural and physiological mechanisms for thermoregulation (Chown and Nicolson, 2004). One

common behavioural mechanism is for an organism to seek a thermally favourable microhabitat. In light of this it is important to ask whether or not the temperature data from which degree-days are accumulated accurately represent the actual temperatures an organism is experiencing (Bonhomme, 2000). Indeed, McClay and Hughes (1995) warn that the use of standard meteorological data for this purpose is a drastic simplification. Furthermore, errors in the collection of temperature data, even if taken directly from these microhabitats, will also lead to inaccurate phenological predictions.

3.1.2 Monitoring environmental temperature

Long-term climatic data must be homogenous, or consistently monitored without error or omission, in order to draw accurate conclusions from any subsequent analyses. Aguilar *et al*. (2003) defines homogenous climatic data as such where any variation within the data is caused only by variations in climate. However, when dealing with real world data sources, one must overcome a number of non-climatic factors that can make data unrepresentative of actual climatic variation. Factors that affect data homogeneity include changes in recording practices, data analysis techniques and monitoring locations or environments; poorly calibrated instrumentation (Aguilar *et al*. 2003); missing data (Kotsiantis *et al*. 2006; Mraoua and Bari, 2007) and instrument exposure (Anderson and Baumgartner, 1998; Brunet *et al*. 2006). Many studies have attempted to develop ways of identifying these non-climatic inconsistencies or inhomogeneities and then develop ways to adjust the data in order to minimise any biases which may be caused. A variety of techniques have emerged regarding these adjustments, which differ widely in terms of method and complexity as necessitated by the specific needs of a particular study or dataset (Aguilar *et al*. 2003). Nevertheless, data homogenisation can be simplified into two main processes of replacing both missing data and erroneous values (Kotsiantis *et al*. 2006).

Complete or continuous temperature datasets are essential for developing accurate and applicable phenological models that are based on degree-days. As a result, a number of filling methods have been developed for reconstructing missing temperature data. These range from naive approaches, such as replacing missing data with values from the same

day of the previous year; to complex algorithms (Mraoua and Bari, 2007) which are normally chosen at the user's discretion. A popular approach to filling missing temperature data is via the construction and subsequent comparison of data to be homogenised with a reference time series of corresponding data. These can be predicted from historical data (Kotsiantis *et al*. 2006) or developed from a similar neighbouring weather station (Aguilar *et al*. 2003; Mraoua and Bari, 2007).

Using neighbouring weather stations or fallback stations in order to replace missing data has become an established standard for the weather industry due to its relative simplicity (Mraoua and Bari, 2007). The fallback method is best suited to filling smaller data gaps (up to 12 consecutive days) and involves calculating an average offset between days from a dataset to be homogenised and a neighbouring or fallback station. The mean difference between these datasets is calculated from 15 days prior to and after the missing sequence. This mean difference can then be used to offset known values from the fallback station in order to fill the missing data sequence (i.e. missing day value = corresponding fallback station day value ± average offset). The fallback method can also be used to fill gaps that are longer than 12 consecutive days. In these cases the average offset is calculated from 15 days prior to the gap, the corresponding missing days themselves and 15 days after the gap from the previous 3 years, preferably of the dataset being homogenised, but data from the fallback station can also be used (Mraoua and Bari, 2007). In a study to assess the accuracy of differing data filling methodologies, Mraoua and Bari (2007) found the fallback method yielded the second most accurate substitute sequence, outperforming other more complex models. A principal components analysis provided more accurate substitute values but required that data be correlated to at least four other complete temperature datasets, which are not always available.

Radiative heating as a result of instrument exposure can be a significant source of error among temperature records (Anderson and Baumgartner, 1998; Berry and Kent, 2005). As a result, complex models incorporating numerous variables have been developed in order to homogenise affected datasets. In an attempt to correct for solar radiative heating errors in ship-based observations of marine air temperatures, Berry and Kent (2005)

developed a model that incorporated the heat budget of a ship. Heating errors were modelled as a function of relative wind speed and the incident solar radiation, thus balancing the radiative heating of the sensor with conductive and convective cooling and the storage of heat. A significantly less complex and more common approach is to construct a reference time series. By employing parallel measurements, for example from shielded and non-shielded temperature sensors, a reference or average offset can be derived in order to homogenise a dataset (Aguilar *et al*. 2003). This technique was employed when adjusting long-term Spanish daily temperature records (Brunet *et al*. 2006). Until the widespread distribution and use of the Stevenson screen, early Spanish records were taken from partially protected open stands that were sensitive to radiative heating. These records were therefore unreliable in terms of maximum temperatures, which were exaggerated as a result of radiative heating. Parallel measurements were then taken using replicas of early open stands and Stevenson screens in order to quantify the warm-bias and determine appropriate average offsets. Maximum temperatures from the older datasets were then adjusted by directly subtracting the monthly estimated offset. In the majority of cases, parallel measurements provided the best means of homogenising data affected by radiative heating. Nevertheless, Aguilar *et al*. (2003) warn that it is important to take these measurements for a period long enough to incorporate the full variability or seasonality inherent in a dataset. Therefore, parallel measurements can frequently cover several years but this duration is often dependant on the needs of the study.

3.1.3 Describing the field scenario

Degree-day models have been extensively used to aid the biological control of arthropod pests but have only rarely been used within weed control systems. Nevertheless, these models have been successfully used to guide the selection of potential natural enemies as well as for the initial selection of suitable release sites. However, due to the labour involved in developing these models, McClay (1996) admits that they are unlikely to become a routine component of any pre-release study, and are thus more appropriate as a post-release research tool. This is especially important when difficulties are encountered in establishing an agent from initial releases or for describing possible population limiting

factors. Within South Africa, degree-day models accurately predicted the field population dynamics of *Stenopelmus rufinasus* Gyllenhal (Curculionidae), a natural enemy released against *Azolla filiculoides* Lamarck (Azollaceae). Field sampling and widespread establishment of the insect confirmed the models' predictions of high generational turnover (Byrne *et al*. 2003). However, failures of these models also exist. The mirid *Eccritotarsus catarinensis* released against water hyacinth in South Africa was predicted to complete between three and 14 generations per year in different regions around the country. Nevertheless *E. catarinensis* failed to establish at a high altitude temperate site where it was predicted to be able to complete up to five generations a year. The mirid was however able to establish at two nearby sites that experience equally low winter temperatures (Byrne *et al*. 2003; Coetzee *et al*. 2007b). This failure to establish was attributed to winter extremes exceeding the insect's thermal tolerance levels. Nevertheless the mirid has been able to establish at other sites that experience similarly low winter extremes (Hill. unpubl.). This dichotomy in survival of the mirid at a number of seemingly similar field sites illustrates the need for fine-scale microclimate temperature data in order to increase the accuracy of subsequent degree-day models as well as determine the level of thermal stress an organism may be exposed to.

Whilst degree-day values accurately portray the amount of heat in a given habitat available for development, they cannot quantify the amount of thermal stress an organism may be subjected to as a result of temperature extremes and stochastic events such as frosting. For instance, nocturnal temperature trends will have a far greater affect on herbivory and reproduction for insects that feed and oviposit at night than daily mean temperature values. Similarly, whilst important in terms of mortality and providing that day time temperatures are favourable, daily minimum temperature would have a negligible impact on the feeding and reproduction of an insect only active during the day. Fine-scale microclimate temperature data is therefore essential to quantify, in terms of accumulating thermal stress, the effects of prolonged exposure to unfavourable minimum temperature extremes, stochastic events such as frosting as well as insect behaviour in terms of activity periods and their implications for feeding, reproduction and mortality.

The aim of this chapter is to describe the seasonal population dynamics of the *Neochetina* weevils, on which very little has been published or is known, and *E. catarinensis* at a variety of water hyacinth infestations encompassing a wide range of South Africa's climate. Degree-day models were constructed based on long-term temperature datasets (up to two years) which were recorded in such a way as to minimise some of the drawbacks associated with the degree-day approach. Hourly temperature data were recorded in order to preserve diurnal trends and three microhabitats were monitored at each site in an attempt to encompass temperatures directly experienced by the different life-history stages of the insects. The homogenisation techniques used to correct radiative heating errors in the temperature data and substitute missing data are also described and critically evaluated in terms of their biological relevance to the insects and their phenology, as well as their potential influence on the models themselves. The acute effects of climate on degree-day based predictions were also investigated. Subsequent predictions based on the phenological degree-day models were also evaluated against long-term, on the ground measurements at each of the field locations.

3.2 Materials and Methods

3.2.1 Monthly monitoring

Field sampling was done at 14 sites throughout South Africa for a period of up to two years. Sites were selected from localities where water hyacinth had been recorded, and which had previously been grouped according to broad climatic conditions (Byrne *et al*. 2010). This grouping of sites was done using Principal Components Analysis on interpolated climatic variables for all known localities where water hyacinth occurs in South Africa (Robertson *et al*. 2004). Specific sites from each group were then selected for monitoring, by considering the status of the water hyacinth population, what agents were present and their population status, and the feasibility of repeated sampling (Table 3.1). Both plant and insect phenological and morphological data were collected at each monitoring site in an attempt to relate these parameters to temperature. Monitoring was done monthly from December 2004 to November 2005 for three of the sites, and until November 2006 for the rest.

Plant dynamics

Monthly sampling recorded plant biomass and density per site calculated from wet weight measures of live above and below water material, and dead material. Plants taken from three 0.5 x 0.5m quadrats placed on a mature water hyacinth mat were counted, weighed and scaled up to determine the total biomass (kg/m^2) and plant density (No. of $plants/m^2$). Dead material was defined as plant tissue which was more than 50% brown, decaying and water logged. Measures from an additional 10 randomly selected plants also recorded the following parameters which were averaged in order to obtain a mean per plant per site for each sample month: Plant phenostage, longest petiole length, maximum root length, length of the second youngest petiole, area of second youngest lamina, and the number of ramets, leaves and flowers per plant. Plant phenostage was characterised as either short (healthy plants less than 15cm high with inflated petioles); medium (15 to 30cm high healthy plants, petioles inflated to attenuating); tall (Plants higher than 30cm, all petioles attenuated); or impacted (short to medium plants with tough spindly petioles and curled laminas). A live leaf was defined as one that was green on at least half of the petiole and could remain upright under its own weight. Petiole lengths were measured from the base at the crown to the base of the lamina and maximum root lengths from the crown to the end of the longest root. Young laminas not yet unfurled were excluded from leaf counts. Ramets were defined as younger plants bearing recognisable leaves and still connected to an adult plant via a stolon. Tracings of lamina circumference made in the field were scanned and used to determine lamina area. Area was calculated from the calibrated digitised circumferences using a purpose written application developed in the program MATLAB.

Insect dynamics

Further destructive sampling of the same 10 plants gave data for *N. eichhorniae*, *N. bruchi*, *E. catarinensis*, *N. albiguttalis*, *O. terebrantis* and the pathogen *C. piaropi*. Due to the rarity of *N. albiguttalis* at the monitoring sites, and the relatively small impact of *O. terebrantis* and *C. piaropi* on the plants, these species were largely ignored from subsequent analyses except where their presence could possibly influence the phenologies of *N. eichhorniae*, *N. bruchi*, and *E. catarinensis*. For *N. eichhorniae* and *N.*

Table 3.1. Geographic data for fourteen water hyacinth infestations in South Africa (see also map pg. 116). Monthly monitoring was conducted at each site between December 2004 and November 2006.

Site Name	Latitude	Longitude	Altitude (m)	n
Eastern Cape				
1. New Years Dam, Alicedale	33º17'38.64"S	26º08'15.71"E	600	24
2. Kubusi River, Stutterheim	32°35'04.55"S	27°28'09.01"E	1000	24
Free State				
3. Feesgronde, Parys	26°52'28.00"S	27°28'50.00"E	1392	12
Gauteng				
4. Farm Dam, North Riding	26º02'36.77"S	27º57'48.51"E	1452	24
5. Delta Park, Randburg	26º07'29.28"S	28º00'39.52"E	1557	24
KwaZulu-Natal				
6. Nseleni River, Richards Bay	28º43'57.01"S	31º59'01.99"E	9	24
7. Hammarsdale Dam, Pietermaritzburg	29º47'59.00"S	30º39'33.00"E	561	24
8. Mbozambo Swamp, Stanger	29º21'45.56"S	31º18'14.55"E	13	24
Limpopo				
9. Mkadhzi Spruit, Kruger National Park	23º49'51.29"S	31º37'56.81"E	222	24
Northern Cape				
10. Warrenton Weir, Warrenton	28 º07'25.00"S	24º56'04.00"E	1196	12
North West				
11. Crocodile River, Brits	25º39'05.27"S	27º47'19.64"E	1108	24
Western Cape				
12. Breede River, Malgas	34°17'16.09"S	20°34'16.85"E	8	24
13. Princess Vlei, Muizenberg	34°02'38.00"S	18°29'09.00"E	10	12
14. Wolseley, Kluitjieskraal	33°26'10.10"S	19°10'33.28"E	250	24

bruchi, adults were separated into species and individuals of each life stage present per plant were counted. The three larval instars were differentiated visually on the basis of size. Larvae could not be visually separated into species. The number of petioles mined, and due to the adult weevil's preference for younger leaves (DeLoach and Cordo, 1976a), the number of feeding scars on the dorsal surface of the 2nd youngest lamina was recorded. Feeding scar counts per leaf were multiplied by monthly leaf production calculated from the standard curve:

$$y = (-0.2836 + 0.0411x)/7 \qquad \text{(Chapter 2)}$$

where y is daily leaf production and x is mean daily water temperature; and averaged for each month. Adult population numbers could then be indirectly estimated via the relationship described by Wright and Center (1984) between the number of weevil feeding scars per leaf and adult density. Estimating weevil population numbers solely by hand collections is often inefficient due to nocturnal adults seeking hiding places at the base of plants during the day which makes them difficult to find, especially when population numbers are very low. Wright and Center (1984) examined data from both the United States and Australia and found a strong relationship between the number of feeding scars and adults present per plant. After slight adjustment to the data due to differing collection techniques, regression analysis yielded almost identical results from the two countries, allowing the data to be grouped, producing a single robust equation:

$$I = 0.0366S^{0.775}$$

where I is the number of weevils per plant and S the number of feeding scars per lamina (Wright and Center, 1984). Adjusted counts of feeding scars per lamina in this study were therefore applied to this formula to provide a second, possibly more robust estimate of adult population density per plant relative to numbers actually observed in the field.

The approximate number of *E. catarinensis* adults per plant was estimated visually at the onset of each field sample to avoid undue disturbance. On the 10 plants the presence or

absence of nymphs was recorded and feeding on the 4th youngest lamina was categorised by ranking the area of tissue damaged into one of five categories: 0%; <5%; 5-25%; 25-50%, 50-100%. *Orthogalumna terebrantis* feeding damage was recorded in a similar way but categorised according to the area of lamina damaged on the 5th youngest leaf as this is where the damage is most obvious. The number of petioles mined by *N. albiguttalis* and the presence or absence of their larvae and pupae, as well as of *C. piaropi*, evidenced by lesions, was also recorded. These measures were averaged over 10 plants to estimate mean insect activity, density and impact per plant per month at each field site.

3.2.2 Recording temperature

Temperature was recorded within three microsites at each field location using Thermochron iButtons (DS1921G; Maxim Dallas Semiconductor Corporation) that have an operational temperature range of between -40°C and 85°C at a resolution of 1°C and a proven accuracy of ±1°C (Hubbart *et al*. 2005). Thermochron iButtons are self contained digital temperature recorders enclosed in a watertight two-terminal stainless-steel can that along with their small size and low cost, make them ideal for measuring the thermal variability of microhabitats (Gasvoda *et al*. 2003). Data were recorded every half hour and averaged hourly. Recordings of air temperature within the water hyacinth canopy, 11cm above the water surface, and water temperature recordings 8cm below the water surface were facilitated by a purpose-built floating iButton housing (Fig. 3.1a). This device held two iButtons, one below the water surface in a watertight but highly conductive brass capsule, and the second in a vented capsule made from engineering grade nylon that has low thermal conductivity and is UV stable. Water hyacinth plants were tethered to the floating device to ensure it remained within the plant canopy. Air temperature was recorded 1.2m above the water surface immediately adjacent to the site via another iButton and housing (Fig. 3.1b) positioned in such a way as to minimise the effect of radiative heating. Due to the relatively short memory capacity (up to 2048 data points), each iButton was replaced monthly in order to preserve the high resolution of data collection.

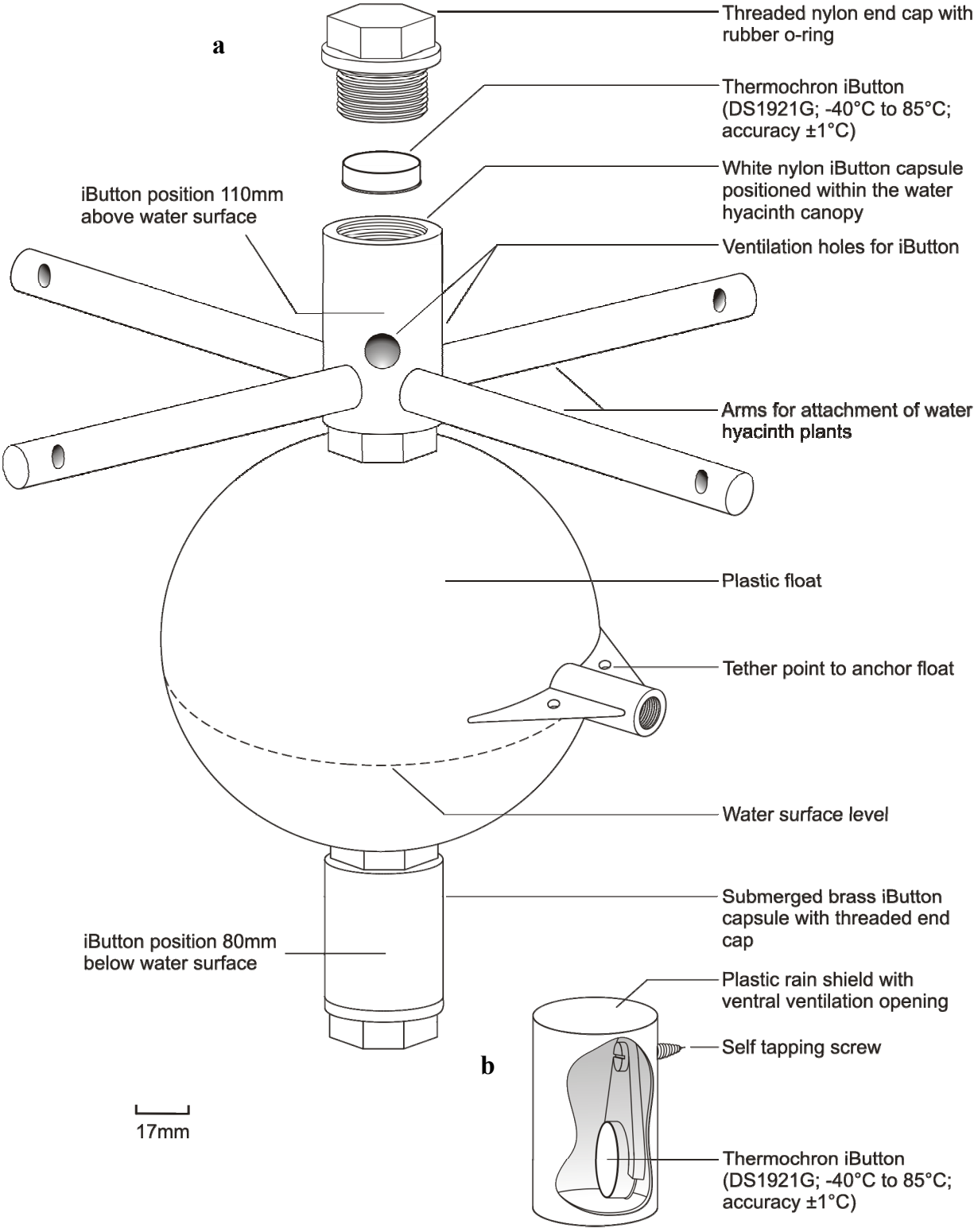


Fig. 3.1. Devices used to monitor temperature (a) within a water hyacinth canopy, water temperature, and (b) air temperature 1.2m above the water surface.

Radiative heating errors

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

In order to preserve the site-specific diurnal trends, data from these tagged periods were corrected by averaging the affected hourly air temperature value with the corresponding hourly water temperature value for the hours between 09H00 and 16H00. Radiative heating errors within the canopy temperature profile were corrected in a similar way except only those months where the mean maximum petiole length was shorter than 15cm were adjusted. Only petioles longer than 15cm were deemed long enough to adequately 'shade' the canopy monitoring iButton capsule from radiative heating. Using water temperature to correct radiative heating errors provided a conservative estimate of 'real' temperatures, negating the need to quantify the relative extent of radiative heating between months at the same site, as well as between sites.

Missing temperature data

Periods of missing data were homogenised using a modified version of the fallback approach described by Aguilar *et al*. (2003). This consisted of using sequential series of reference time series, or reference datasets, to first fill missing data within the air

temperature and then the canopy temperature profiles. As with the reference time series used to correct radiative heating errors, missing air temperature values were derived from offset values obtained from the closest available weather station. Due to the high number of missing data sequences longer than 12 consecutive days, average offsets were calculated from the longest period, but not exceeding a year, of 'complete' data available and not only from 15 days prior to and after the gap as is done in the fallback method described earlier. The offset was calculated as the mean difference between daily air temperature means calculated from microsite data and those calculated from the corresponding period of weather station data. Weather station data only included a daily maximum and minimum temperature so daily means were calculated from these extremes. The average offset was then directly added to or subtracted from the corresponding value obtained from the weather station data and substituted into the missing sequence of microsite air temperature data. To preserve a complete sequence of hourly temperature recordings for microsite data for later degree-day calculation, this corrected daily mean value was substituted for each hour of the relevant day. Following the homogeneity adjustments to the air temperature profile, an average offset between this dataset and the canopy temperature profile was calculated in the same way. Missing values from the canopy temperature dataset could then be filled using offset values derived from the air temperature profile and inputted in the same manner as before. This modified fallback approach was applied to all missing data sequences longer than 24 hours. Gaps shorter than this were filled using the linear trend between the observed values on either side of the gap.

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

3.2.3 Degree-day accumulation

To preserve the diurnal trends within the temperature data, degree-day accumulation was calculated from hourly data. Heat units for the *Neochetina* weevils and *E. catarinensis* were calculated by subtracting the relevant developmental threshold from the canopy temperature recorded for each hour of the diurnal cycle. These values were then summed and divided by 24 to express hourly thermal units on a daily basis as expressed by the following formula:

$$°\mathrm{D} = \left(\sum_{h=1}^{24} T - t \right) / 24 \qquad \text{(Purcell, 2003)}$$

where T is the hourly temperature value and t is the developmental threshold temperature. Daily degree-days were summed for each site and averaged per year. The numbers of generations per year were estimated for the *Neochetina* weevils and *E. catarinensis* by dividing their thermal constants (K) by the total yearly degree-day accumulations.

In order to quantify the error associated with filling missing temperature sequences with offset weather station data as described above, degree-days derived from actual canopy microsite datasets were compared with degree-days derived from twice offset weather station data. Twice offset data refers to missing data periods from both the air and canopy temperature profiles. This provided a biologically meaningful site-specific degree of error based on the level of agreement between the estimates made from each dataset multiplied by the mean number of gaps per year and the average gap duration. The level of agreement was calculated as the mean difference $\bar{d}$ between daily degree-day estimates from canopy microsite data and those derived from corresponding weather station data and where 95% of differences lie between $\pm 1.96s$. s is the standard deviation of the differences as per the methodology described by Bland and Altman (1986). The longest non-filled sequence of canopy data available, not used for the development of the original offset used to fill missing values and not exceeding a year, was used to calculate the mean difference between offset data and actual field data, if it had been recorded. The mean difference was multiplied by the mean number of gaps per year and the average gap duration (days) to obtain a measure of the yearly mean error (°D/year).

3.2.4 Stress indices

A number of climatically based 'stress indices' similar to those developed by Bezuidenhout *et al*. (2008) were created using thermal thresholds derived in chapter two for the *Neochetina* weevils, *E. catarinensis* and water hyacinth in order to characterise and compare field sites by the amount of accumulating thermal stress. These take into account the effects of prolonged exposure to unfavourable minimum temperature extremes, stochastic events such as frosting as well as insect behaviour in terms of activity periods, and their implications for feeding, reproduction and mortality. This is done by quantifying the amount perceived stress, defined by a set of stress indices, on the organism through counts of unfavourable temperatures and events.

Neochetina *weevil stress indices*

Oviposition index: The number of nocturnal hours (between 19H00 and 05H00) per annum with a mean hourly canopy temperature below the oviposition threshold (12.5°C); Developmental index: The maximum number of consecutive days each year with a mean daily canopy temperature below the lower developmental threshold (9.6°C); Feeding index: The number of days per annum with a mean nocturnal canopy temperature below which feeding ceases (6.3°C); and a Mortality index: The maximum number of consecutive days each year with a daily minimum canopy temperature below the CT_{min} temperature (3.8°C).

Eccritotarsus catarinensis *stress indices*

Oviposition index: The number of diurnal hours (between 05H00 and 19H00) per annum with a mean hourly canopy temperature below the oviposition threshold (10.8°C); Developmental index: The maximum number of consecutive days each year with a mean daily canopy temperature below the lower developmental threshold (10.3°C); Feeding index: The number of days per annum with a mean diurnal canopy temperature below which feeding ceases (10.8°C); and a Mortality index: The maximum number of consecutive days each year with a daily minimum canopy temperature below the CT_{min} temperature (1.2°C).

Eichhornia crassipes *stress indices*

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

Index values were averaged from two years of temperature data (Dec 2004 – Nov 2006) where available. These means were added together to provide a relative overall score of thermal stress for each species at each site to enable comparisons between sites. To standardise the contribution of each index to the overall score, annual hourly counts were divided by 365. Principal components analysis of all stress index values was used to group sites with similar stress magnitudes.

3.3 Results

3.3.1 Radiative heating

Air temperature data from three sites were adjusted for radiative heating errors. Plotting of hourly data clearly showed aberrant readings within the air temperature profile enabling periods with excessively high temperature values to be readily identified (Fig. 3.2) and tagged for adjustment. Two periods from New Years Dam of 126 (2005/12/26 to 2006/04/30) and 92 days (2006/08/31 to 2006/11/30) respectively were corrected. One period of 69 days (2004/12/01 to 2005/02/07) was adjusted from the Warrenton Weir site and two periods (2004/12/08 to 2005/01/08 and 2006/01/30 to 2006/03/01[Fig. 3.2]) each lasting 31 days were adjusted from the Wolseley site. Daily maximum temperatures were found to be significantly higher than the corresponding offset weather station data during all periods adjusted for radiative heating. To validate the method of adjusting aberrant air temperature values using the water temperature profile, maximum and mean daily air temperature from the original, adjusted and corresponding offset weather station datasets was compared. Figs 3.3a and 3.3b show maximum and mean daily air temperature data respectively, recorded for the same duration at Warrenton Weir during periods affected (i and iii) and unaffected (ii and iv) by radiative heating.

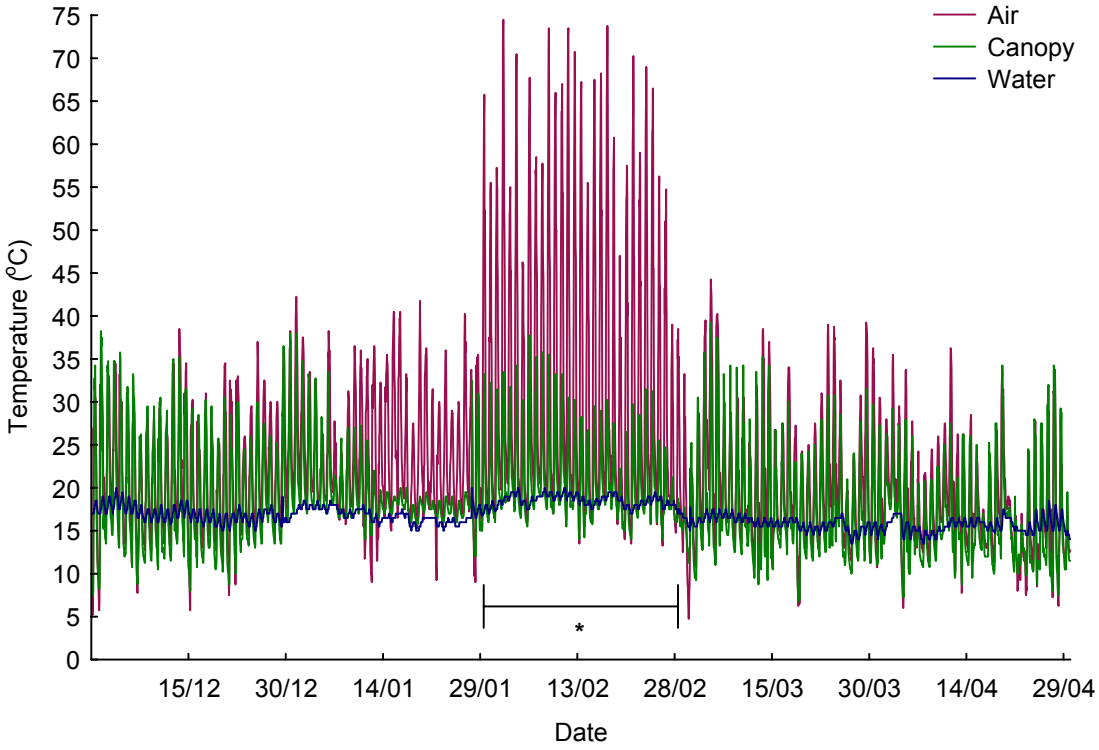


Fig. 3.2. An example of hourly temperature data from the Wolseley field site for three microsites showing aberrant readings (*) in the air temperature profile caused by radiative heating of the temperature sensor.

Following adjustment, maximum ($F_{2, 201} = 54.49$; $P < 0.001$) and mean ($F_{2, 201} = 9.51$; $P < 0.001$) daily air temperatures did not differ significantly from the corresponding offset weather station data even though the original datasets contained significantly warmer values (Fig. 3.3: graph inserts). Original maximum and mean daily temperature recordings showing radiative heating were on average 7.7°C and 1.1°C warmer than the corresponding offset weather station data respectively. The mean difference between adjusted air temperature values and the corresponding weather station values was also compared with the mean difference between original data unaffected by radiative heating and weather station data directly following the period of adjustment and lasting for the same duration. Unfortunately, the mean difference (1.3 ±0.5°C) between adjusted daily maximum temperatures and daily maxima from offset weather station data (Fig. 3.3: period i) was significantly less ($t = -2.03$; $P = 0.04$; d.f. = 134) than the mean

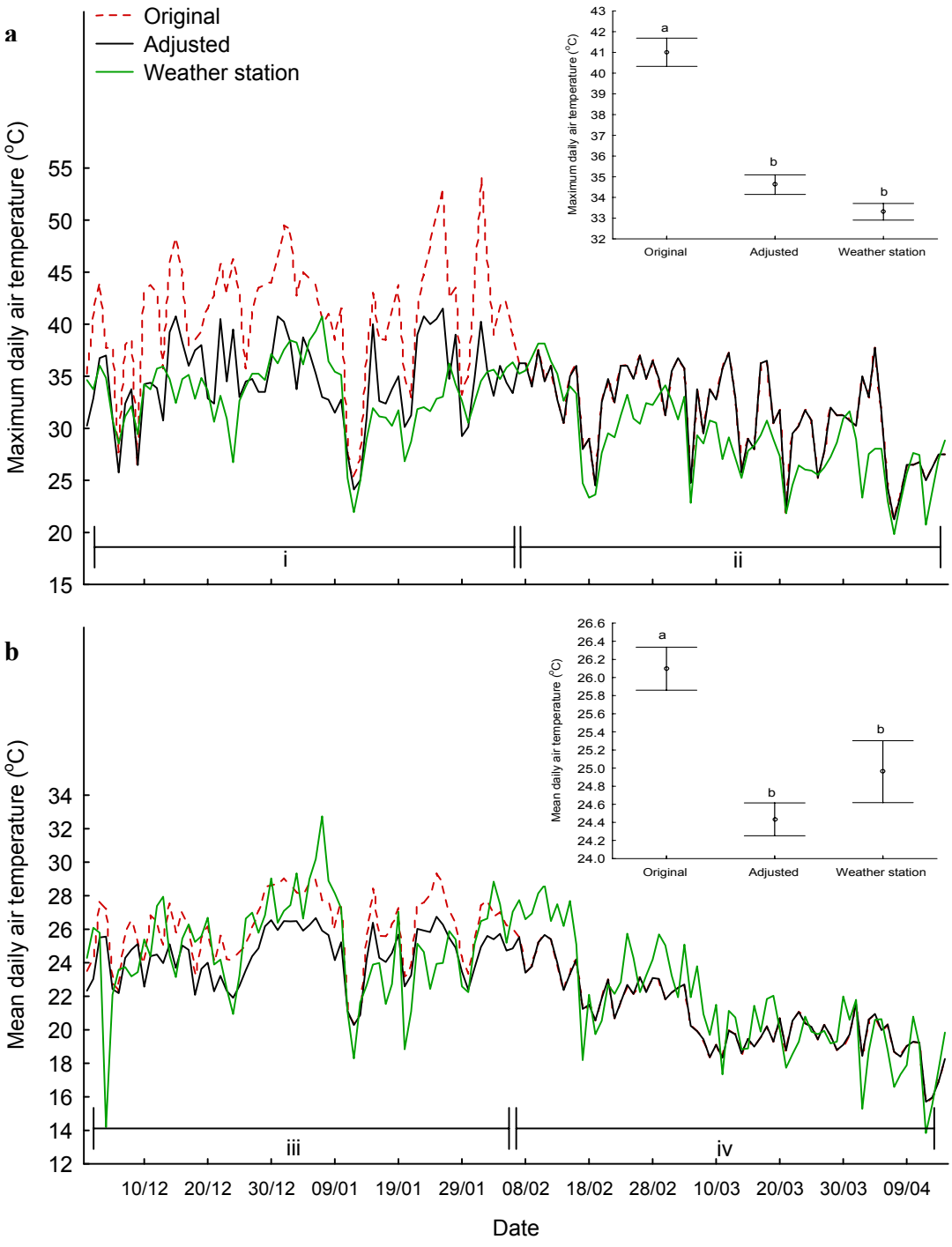


Fig. 3.3. Daily maximum (a) and daily mean (b) air temperature for periods affected (i, iii) and unaffected (ii, iv) by radiative heating of the temperature sensor (2004 – 2005) at Warrenton Weir. Graph inserts show the relative mean (±SE) values for the original, adjusted and reference time series datasets over periods i and iii respectively. Data with the same letters are not significantly different at $P < 0.05$ (ANOVA, Tukey HSD test).

difference (2.6 ±0.4°C) between adjacent unadjusted data and weather service data (Fig. 3.3: period ii). Nevertheless, these maximum values were restricted to a few hours a day and their subsequent contribution to the diurnal trend and degree-day accumulation is therefore small. Consequently, the mean difference (-0.5 ±0.3°C) between adjusted daily mean temperature values and the corresponding weather station values (Fig. 3.3: period iii) was not significantly different ($t = 0.53$; $P = 0.59$; d.f. = 134) from the mean difference (-0.7 ±0.2°C) between adjacent unadjusted and weather station means (Fig. 3.3: period iv).

Despite the good association between adjusted air temperature values and offset weather station data at Warrenton Weir, in some cases radiative heating was so excessive that although greatly reduced, adjusted data were still significantly higher than the corresponding weather station data. At New Years Dam between 2006/08/31 and 2006/11/30 adjusted mean daily temperatures were still significantly higher ($t = 2.53$; $P = 0.00$; d.f. = 180), on average 2.1 ±0.3°C, warmer than the offset weather station data. Both periods adjusted at Wolseley also remained significantly higher, 1.4 ±0.4°C between 2004/12/08 and 2005/01/08 ($t = 2.32$; $P = 0.02$; d.f. = 62), and 2.2 ±0.5°C between 2006/01/30 and 2006/03/01 ($t = 3.09$; $P = 0.00$; d.f. = 60) than the original data.

Due to the relatively low thermal conductivity of the nylon capsule on the floating iButton housing, canopy temperature values were not inflated to the same extent as that observed from air temperature profiles. From a separate pilot study, iButtons exposed to full sunlight recorded values on average 9.4 ±0.1°C higher than an adequately screened nylon iButton capsule. By comparison, capsules exposed to full sunlight were on average only 4.1 ±0.1°C higher than the screened capsule. Therefore, periods during which canopy temperature sensors were exposed to radiative heating could not be readily distinguished or demarcated from plots of the temperature data. Nevertheless, uncharacteristically high temperature values were evident and predominantly associated with small plants as a result of leaf senescence during winter or disturbance, for example after flooding when sites are dominated by smaller colonising plants.

Forty five out of a total of 300 months among all the sites were adjusted for radiative heating errors associated with the canopy temperature profiles during the two years of monitoring. Delta Park contained the most radiative heating errors, requiring 13 months of canopy data, during and directly following winter from both years, to be adjusted. Small plants prevalent at these times were most likely the result of heavy frosting causing laminas to brown and die resulting in decreased petiole lengths. Furthermore, low water nutrients at the site (Byrne *et al*. 2010) meant that plants were slow to recover resulting in smaller average plant size during the months following winter. The greatest number of consecutive months adjusted occurred at Delta Park where seven months of canopy data were adjusted from July 2005 to January 2006. No adjustments to the canopy temperature profiles at Crocodile River, Hammarsdale Dam or Wolseley were made. Of the remaining 10 sites, on average 2.05 ±0.61 months per year per site were adjusted for radiative errors.

The method of adjusting canopy temperature data from the water temperature profile was tested in the same way as that applied to corrected air temperature data. However, in this case offset values from the corresponding air temperature profile, including adjusted portions were used for comparison instead of weather station data. Figs 3.4a and 3.4b show maximum and mean daily canopy temperature data respectively, recorded for the same duration at Delta Park during four months where mean petiole length was less than (Fig. 3.4: periods i and iii) and greater than (Fig. 3.4: periods ii and iv) 15cm. Adjusted maximum daily canopy temperatures were still significantly higher than the offset air temperature values although they were significantly reduced compared with the original data set ($F_{2, 273} = 118.01$; $P < 0.001$) (Fig. 3.4a: graph insert). As before, daily maxima had seemingly little impact on the diurnal trend and consequently adjusted mean daily canopy values were not significantly different from offset air temperature data even though the original canopy dataset was significantly warmer ($F_{2, 273} = 24{,}53$; $P < 0.001$) (Fig. 3.4b: graph insert).

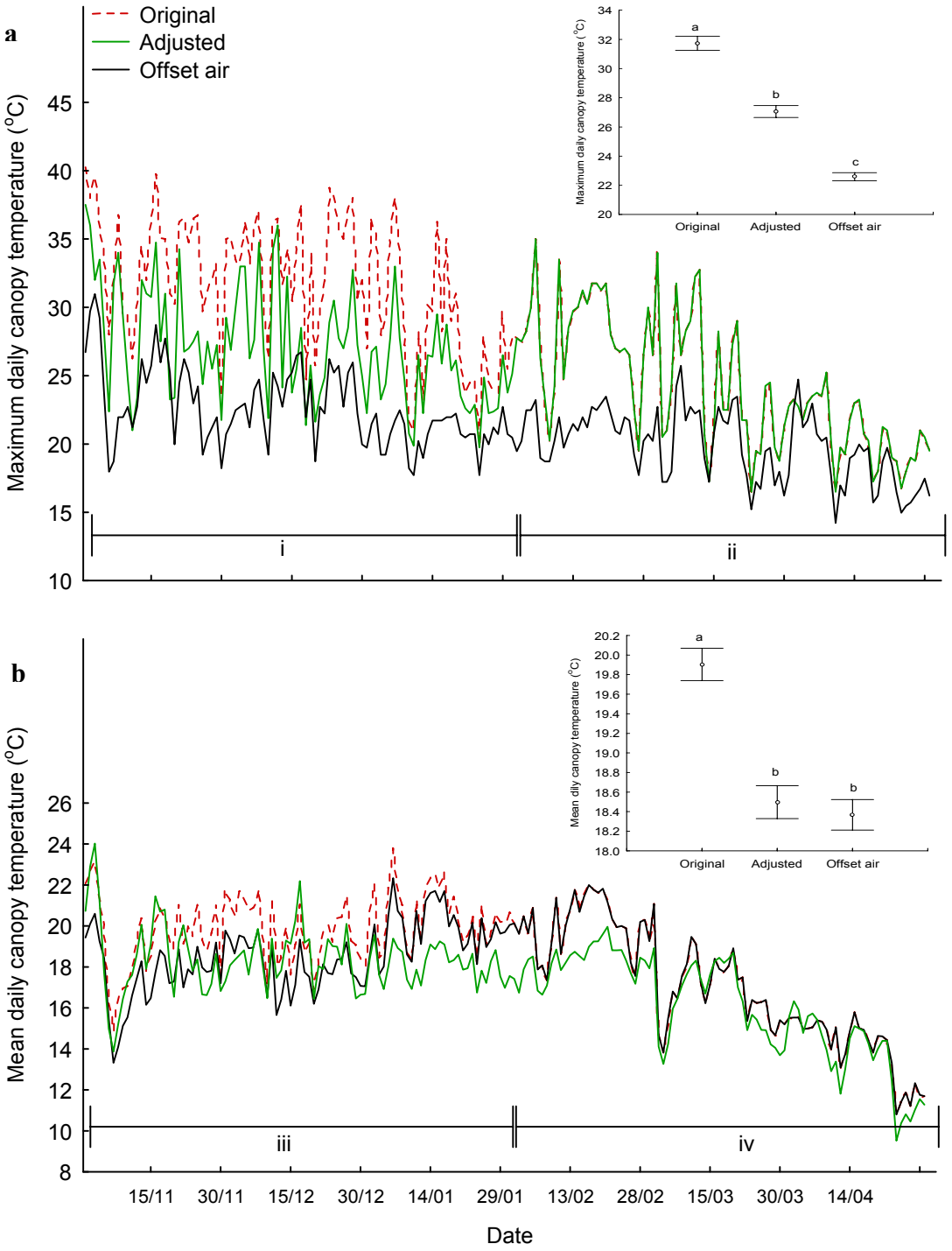


Fig. 3.4. Daily maximum (a) and daily mean (b) canopy temperature for periods affected (i, iii) and unaffected (ii, iv) by radiative heating of the temperature sensor (2005 – 2006) at Delta Park. Graph inserts show the relative mean (±SE) values for the original, adjusted and reference time series datasets over periods i and iii respectively. Data with the same letters are not significantly different at $P < 0.05$ (ANOVA, Tukey HSD test).

Original maximum and mean daily canopy temperature recordings from sensors subjected to radiative heating were on average 9.1°C and 1.5°C greater than the corresponding offset air temperature data respectively. The mean difference between adjusted data and offset air temperature data between periods affected and unaffected by radiative heating was also analysed. The mean difference (4.5 ±0.3°C) between adjusted daily and offset air temperature maxima (Fig. 3.4, period i) was not significantly different (t = -0.11; P = 0.91; d.f. = 179) to the difference (4.4 ±0.3°C) between unadjusted canopy data and offset air temperature data (Fig. 3.4: period ii). Conversely, the mean difference (0.1 ±0.2°C) between adjusted daily and offset air temperature means (Fig. 3.4: period iii) was significantly less (t = 4.00; P = 0.00; d.f. = 179) than the difference (1.0 ±0.1°C) between unadjusted canopy data and offset air temperature data (Fig. 3.4: period iv). Thus, in this case at least, adjusted means appear to approximate more closely the broader overall trend prevalent at the site. Although the adjustment of the canopy temperature profile at Delta Park closely approximated the offset air temperature dataset in terms of daily means, and as such the subsequent calculation of degree-days would not be significantly altered from what was deemed correct, this did not hold true for all periods adjusted. In some instances adjusted daily means were significantly colder and in other cases remained significantly warmer than expected.

3.3.2 Degree-day accumulation and associated error

Unbroken temperature datasets are essential for degree-day summation and thus, to predict insect phenology accurately. Unfortunately, missing sequences of data were unavoidable for a variety of reasons ranging from sensor malfunctions, although this was limited to only five iButtons, to the loss of iButton housings due to flooding and theft. Of the 14 sites monitored, only two sites, Delta Park and Farm Dam, had unbroken records for all three microsites spanning the entire two year sampling period (Fig. 3.5). Nevertheless, only minor gaps were recorded at Crocodile River, Feesgronde, Hammarsdale Dam, Mbozambo Swamp, Mkadhzi Spruit, Nseleni River and Warrenton Weir having between them on average 1.55 ±0.36 gaps per year with a mean duration of 9.77 ±2.45 days absent from all three microsites. The most incomplete datasets were recorded at Breede River with 3.50 ±0.00 gaps per year with a mean duration of 56.05

±1.81 days per gap, and Princess Vlei with 4.30 ±0.33 gaps per year with a mean duration of 48.12 ±9.60 days, averaged from the three microsites. Kubusi River (3.67 ±0.17 gaps/year of 38.39 ±2.76 days/gap), New Years Dam (4.00 ±0.28 gaps/year of 31.38 ±2.25 days/gap), and Wolseley (4.50 ±0.00 gaps/year of 16.67 ±0.00 days/gap) were also affected by missing sequences of data although to a lesser extent. Two year temperature datasets were collected at most sites but due to logistical reasons, only one years worth of data was recorded at Feesgronde, Princess Vlei and Warrenton Weir.

Homogenisation of the canopy temperature data using the modified fallback approach enabled estimates of the number of insect generations to be calculated from accumulated degree-days at all sites with minimal error defined by the mean difference (Tables 3.2 and 3.3). The highest mean error caused by homogenisation occurred for Princess Vlei which accumulated an error of ±0.35 *Neochetina* (Table 3.2), and ±1.00 *E. catarinensis* (Table 3.3) generations per year. By comparison, the other most incomplete dataset recorded for Breede River returned a relatively small error of ±0.03 *Neochetina*, and ±0.11 *E. catarinensis* generations per year. This unexpectedly low error was due to the very small mean difference of 0.2 ±3.7 degree-days per day for the *Neochetina* weevils (Table 3.2) and 0.2 ±3.6 degree-days per day for *E. catarinensis* (Table 3.3) which was afforded by the good fit of the reference time series. The best estimates were obtained for Delta Park and Farm Dam as a result of their unbroken datasets. The most generations observed per year were recorded at Mkadhzi Spruit and Nseleni River which were consistently warmer, accumulating 5.03 ±0.01 and 4.74 ±0.03 *Neochetina*, and 13.77 ±0.01 and 12.93±0.09 *E. catarinensis* generations per year respectively. In contrast, the coldest site was Kubusi River which accumulated only 2.08 ±0.22 *Neochetina* and 5.38 ±0.62 *E. catarinensis* generations per year.

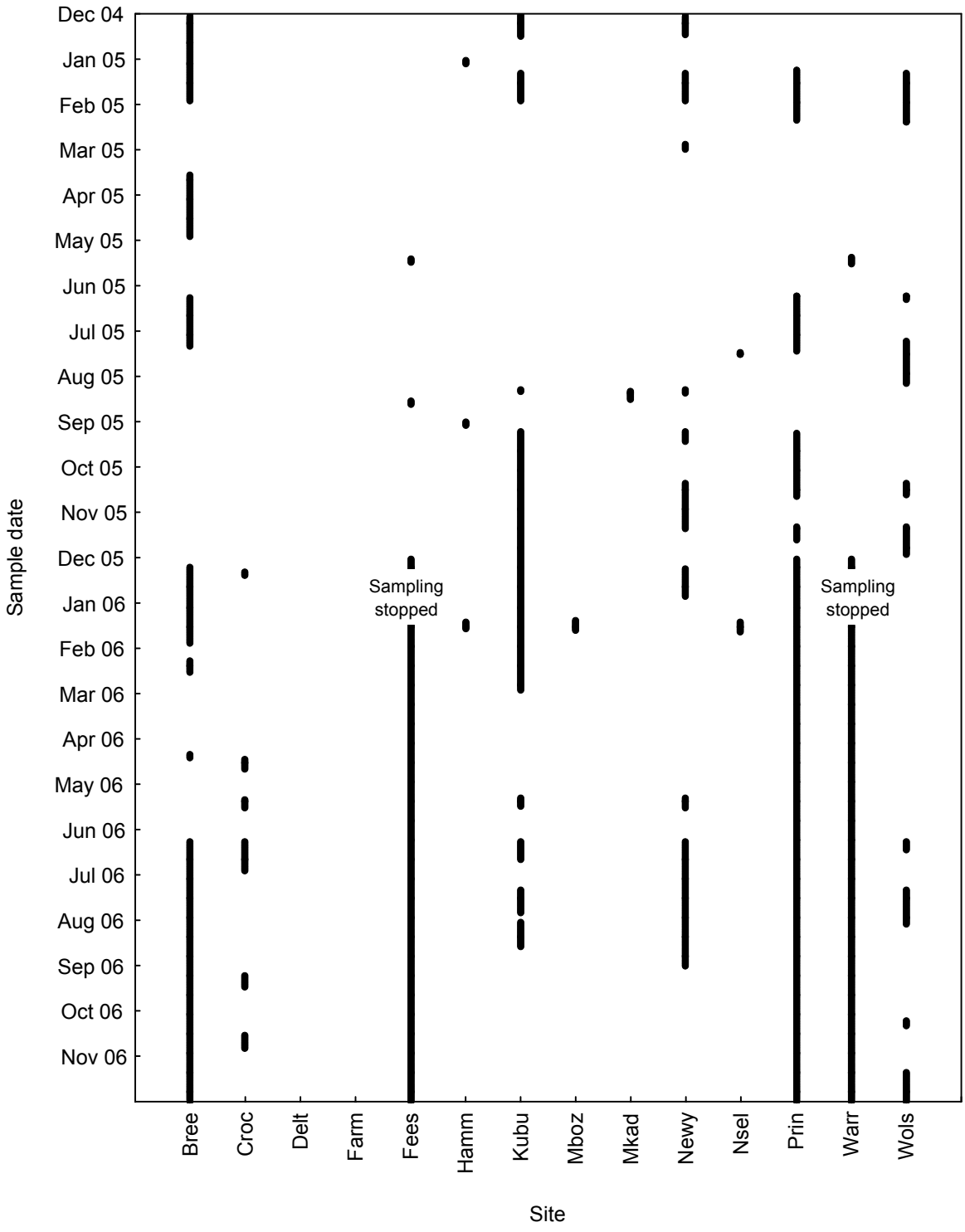


Fig. 3.5. Periods of missing temperature data (black lines) from all three microsites concurrently (water, canopy and air temperature profiles) during two years of monitoring at 14 water hyacinth infested sites distributed around South Africa.

Table 3.2. Number of *Neochetina* generations per year at 14 water hyacinth infested sites distributed around South Africa when calculated from incomplete sequences of canopy temperature data (developmental zero of 9.6°C). Yearly mean error in the accumulation of degree-days was calculated as the product of the number of data gaps, the length of these gaps and the mean difference between actual canopy data and a reference time series derived from offset regional weather station data.

Site name	Number of gaps filled ($year^{-1}$)	Mean gap length (days/year ±SE)	Mean difference (°D/day)	Accumulated degree-days (°D/year ±error)	Number of generations ($year^{-1}$ ±error)
Breede River	3.5	57.62 ±26.63	0.2 ±3.7	2738.2 ±30.3	2.78 ±0.03
Crocodile River	3.5	12.58 ±2.92	1.4 ±4.2	3041.9 ±63.4	3.09 ±0.06
Delta Park	0	-	-	2152.1	2.19
Farm Dam	0	-	-	2324.5	2.36
Feesgronde	2	2.50 ±0.00	-1.3 ±3.5	3174.2 ±6.6	3.22 ±0.007
Hammarsdale Dam	2	4.50 ±0.29	1.2 ±2.9	2968.9 ±10.7	3.02 ±0.01
Kubusi River	4	38.47 ±19.94	1.4 ±3.4	2044.0 ±218.5	2.08 ±0.22
Mbozambo Swamp	0.5	4.00 ±0.00	0.4 ±3.5	4325.2 ±0.9	4.39 ± 9^{-4}
Mkadhzi Spruit	1	23.00 ±0.00	-0.2 ±3.5	4953.4 ±4.8	5.03 ±0.005
New Years Dam	4.5	39.75 ±18.67	-0.3 ±7.6	3109.1 ±53.7	3.16 ±0.05
Nseleni River	1.5	16.00 ±0.00	1.3 ±3.2	4669.6 ±30.2	4.74 ±0.03
Princess Vlei	4	61.75 ±24.61	-1.4 ±5.7	3264.1 ±343.3	3.32 ±0.35
Warrenton Weir	1	5.00 ±0.00	0.5 ±5.5	2985.4 ±2.3	3.03 ±0.002
Wolseley	4.5	16.38 ±5.34	-0.2 ±5.5	2548.4 ±11.1	2.59 ±0.01

Table 3.3. Number of *Eccritotarsus catarinensis* generations per year at 14 water hyacinth infested sites distributed around South Africa when calculated from incomplete sequences of canopy temperature data (developmental zero of 10.3°C). Yearly mean error in the accumulation of degree-days was calculated as the product of the number of data gaps, the length of these gaps and the mean difference between actual canopy data and a reference time series derived from offset regional weather station data.

Site name	Number of gaps filled ($year^{-1}$)	Mean gap length (days/year ±SE)	Mean difference (°D/day)	Accumulated degree-days (°D/year ±error)	Number of generations ($year^{-1}$ ±error)
Breede River	3.5	57.62 ±26.63	0.2 ± 3.6	2506.7 ±36.3	7.33 ±0.11
Crocodile River	3.5	12.58 ±2.92	1.5 ± 4.0	2836.6 ±66.5	8.29 ±0.19
Delta Park	0	-	-	1972.4	5.77
Farm Dam	0	-	-	2142.9	6.27
Feesgronde	2	2.50 ±0.00	-1.1 ± 3.5	2971.3 ±5.6	8.69 ±0.02
Hammarsdale Dam	2	4.50 ±0.29	1.3 ± 2.7	2753.3 ±11.3	8.05 ±0.03
Kubusi River	4	38.47 ±19.94	1.4 ± 3.3	1840.0 ±210.8	5.38 ±0.62
Mbozambo Swamp	0.5	4.00 ±0.00	0.5 ± 3.5	4085.4 ±1.0	11.95 ±0.003
Mkadhzi Spruit	1	23.00 ±0.00	-0.2 ± 3.4	4709.3 ±4.4	13.77 ±0.01
New Years Dam	4.5	39.75 ±18.67	-0.2 ± 7.3	2883.7 ±34.0	8.43 ±0.10
Nseleni River	1.5	16.00 ±0.00	1.3 ± 3.2	4421.4 ±30.2	12.93 ±0.09
Princess Vlei	4	61.75 ±24.61	-1.4 ± 5.7	3016.0 ±343.3	8.82 ±1.00
Warrenton Weir	1	5.00 ±0.00	0.5 ± 5.4	2790.7 ±2.7	8.16 ±0.008
Wolseley	4.5	16.38 ±5.34	-0.1 ± 5.5	2336.0 ±9.6	6.83 ±0.03

3.3.3 Microclimate data

The temperature profiles (Figs 3.6 - 3.19a) at all sites were extremely variable both within sites in terms of seasonality, as well as between sites. Seasonal variability was greatest at Farm Dam which recorded a difference of 12.5°C between the average summer and winter mean canopy temperatures. Of the 5 most variable sites, all occurred on the highveld above an altitude of 1000m and included Farm Dam, Delta Park (11.4°C), Crocodile River (11.3°C), Feesgronde (11.2°C) and Warrenton Weir (12.1°C). Princess Vlei (8.1°C) and Nseleni River (8.2°C) were the least seasonal, both of which were in coastal areas and therefore presumably subjected to the ameliorating effects of coastal weather systems. Delta Park and Farm dam recorded cold mean winter canopy temperatures of 8.0 and 8.1°C respectively, and Kubusi River was the coldest site, accumulating the least number of degree-days per year, but having a mean winter temperature of 10.0°C, and an 8.4°C difference between the average summer and winter mean temperatures. Mkadhzi Spruit and Nseleni River were the warmest sites having summer mean canopy temperatures of 27.7 and 26.5°C respectively, 9.4 and 8.2°C higher than the lowest summer mean of 18.4°C recorded at Kubusi River.

Free from the influences of radiative heating, minimum canopy and air temperatures tracked water temperature closely at all sites although with more variability in terms of extremes. This variability was greatest at New Years Dam (Fig. 3.15a), Princess Vlei (Fig. 3.17a) and Wolseley (Fig. 3.19a). In addition to this, minimum canopy temperatures were most often warmer or minimum extremes buffered relative to air temperature. This relationship was prominent throughout sites but most evident at Breede River (Fig. 3.6a), Crocodile River (Fig. 3.7a), Hammarsdale Dam (Fig. 3.11a), Kubusi River (Fig. 3.12a), New Years Dam and Princess Vlei. However, in some cases this was true for only part of the time. At Mbozambo Swamp, minimum canopy temperatures appeared to be buffered relative to minimum air temperatures during winter 2005 but not during winter 2006 (Fig. 3.13a). A similar relationship was evident at Mkadhzi Spruit where the two minimum temperature profiles oscillated around each other, alternating between periods of buffered and non-buffered canopy temperatures (Fig. 3.14a). Similarly, during summer at

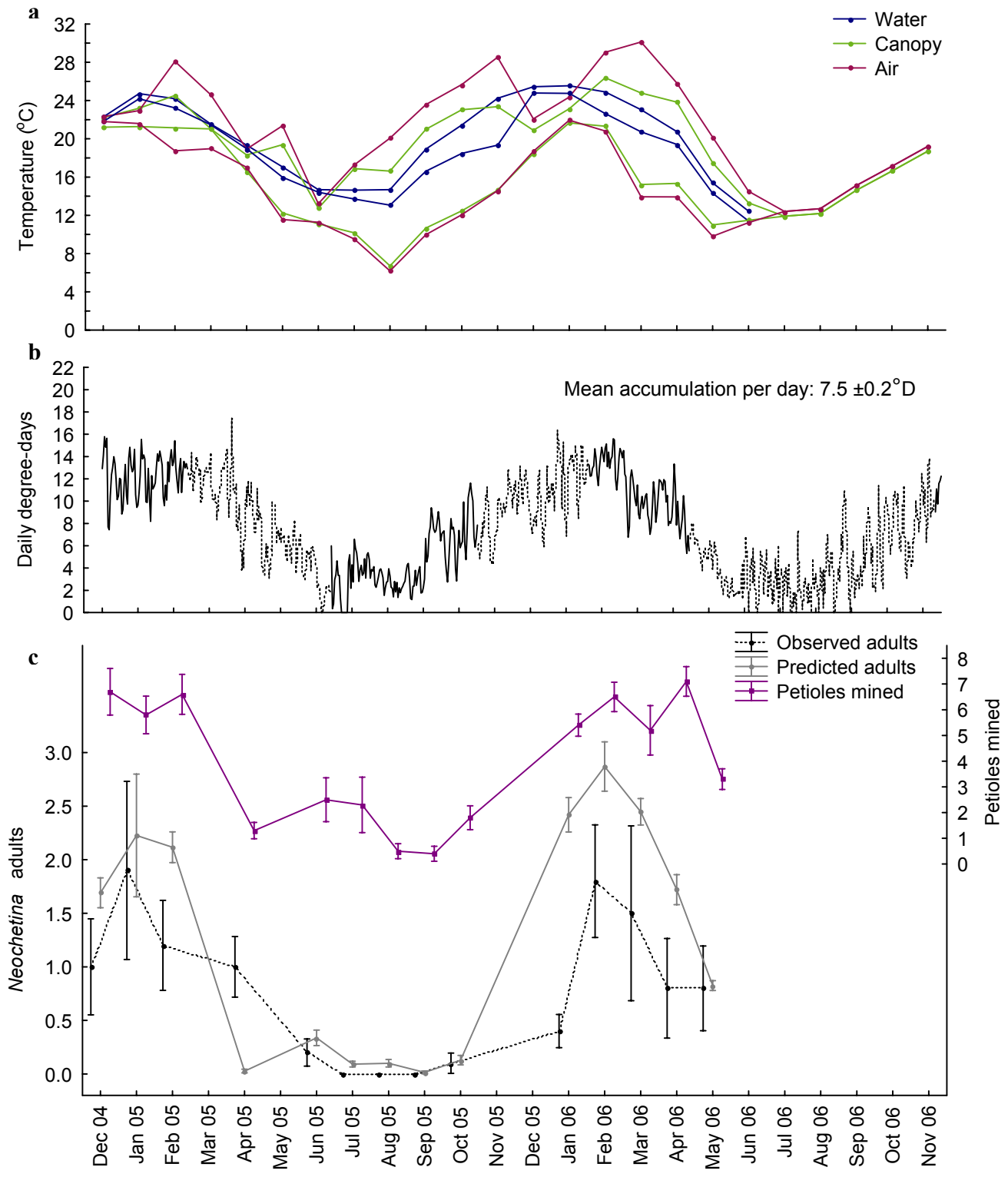


Fig. 3.6. Breede River: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

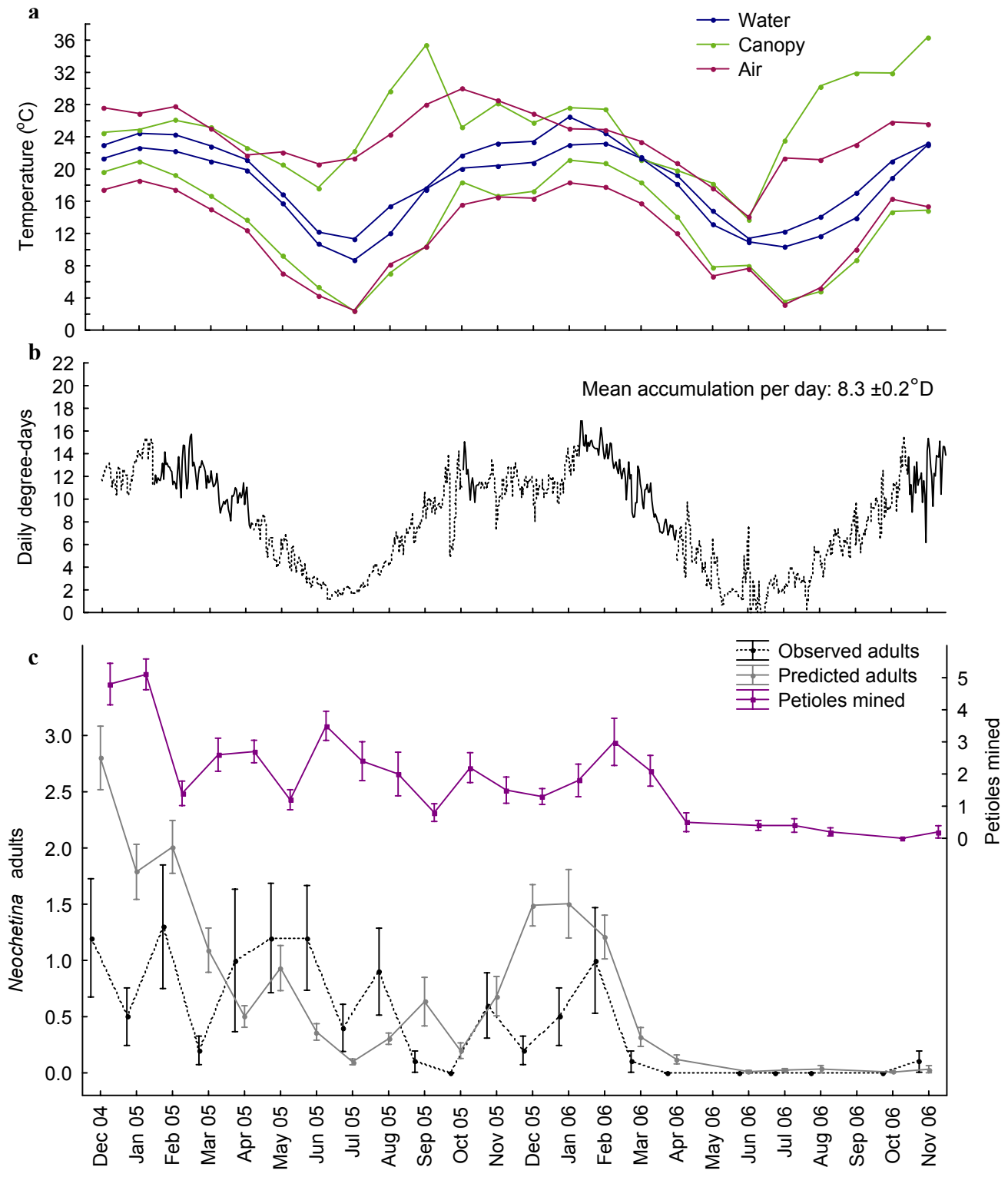


Fig. 3.7. Crocodile River: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

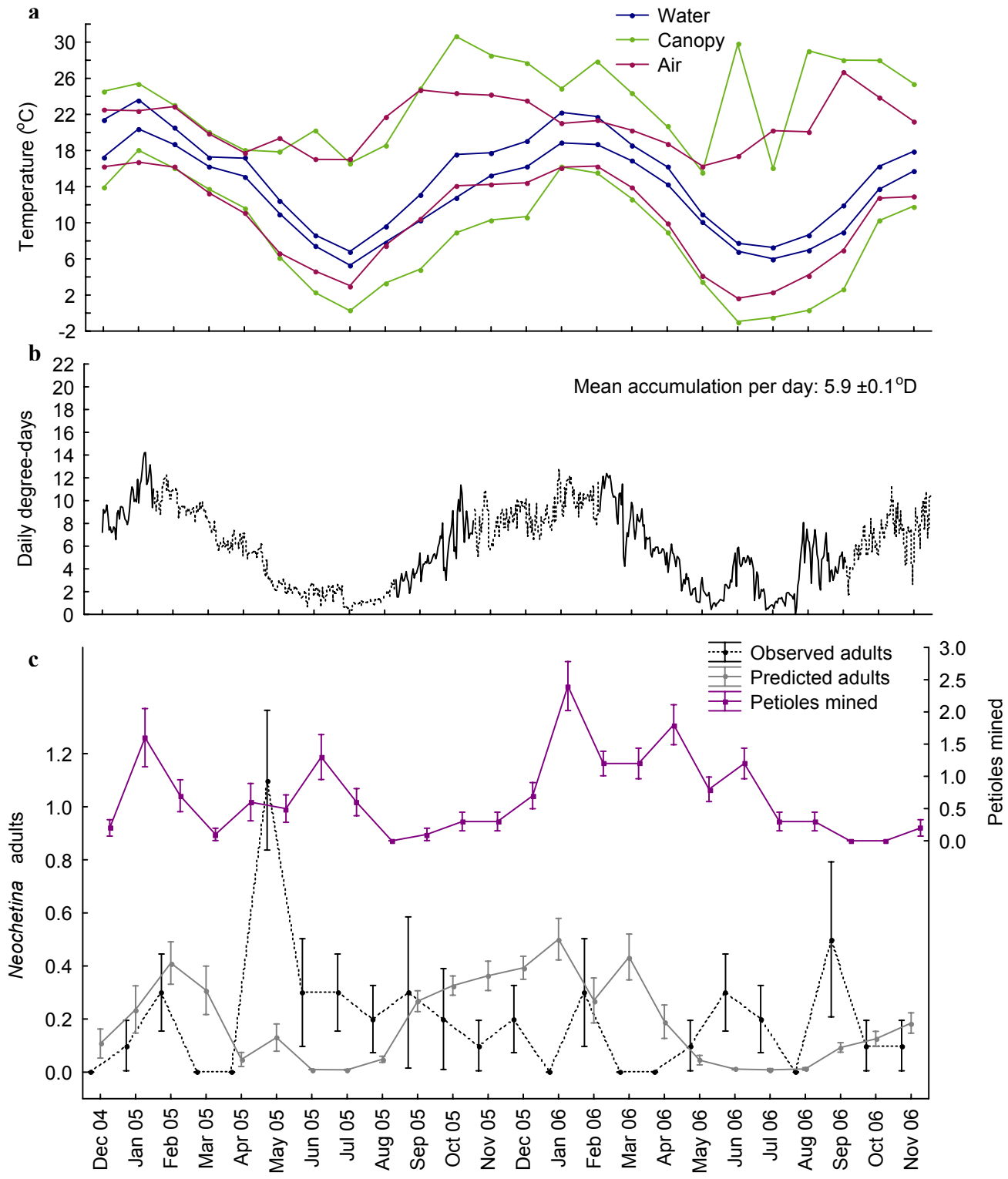


Fig. 3.8. Delta Park: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

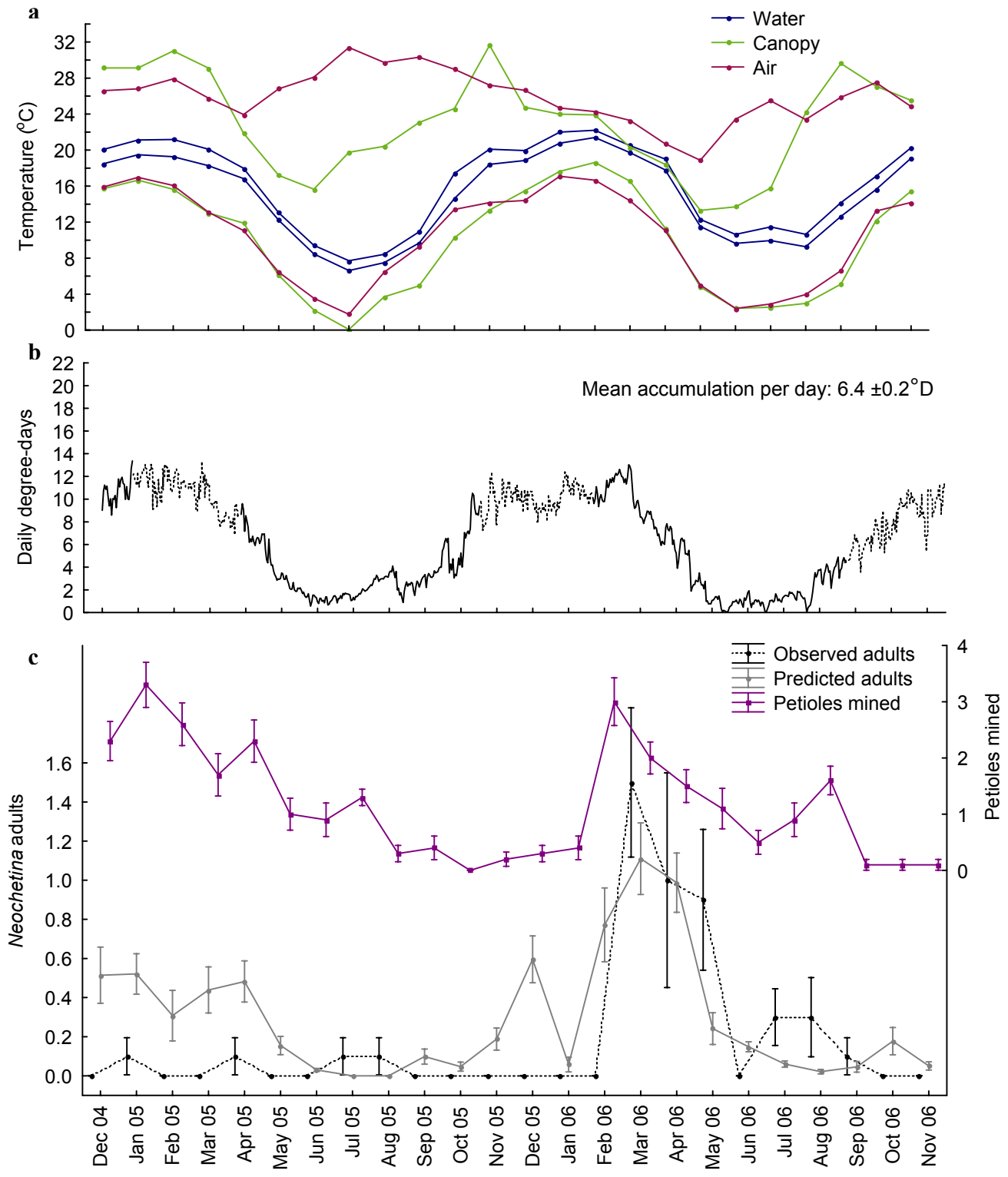


Fig. 3.9. Farm Dam: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

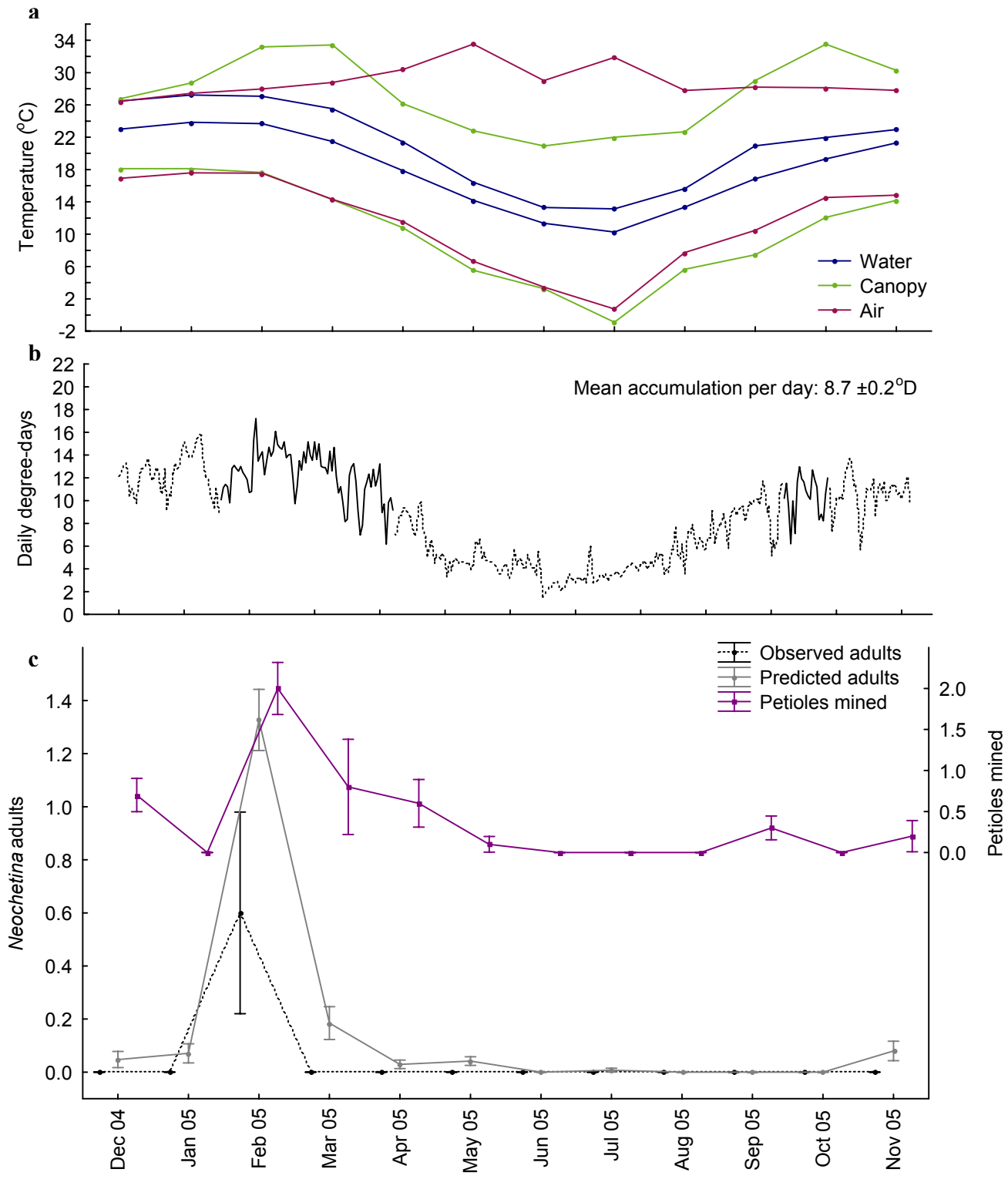


Fig. 3.10. Feesgronde: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2005. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

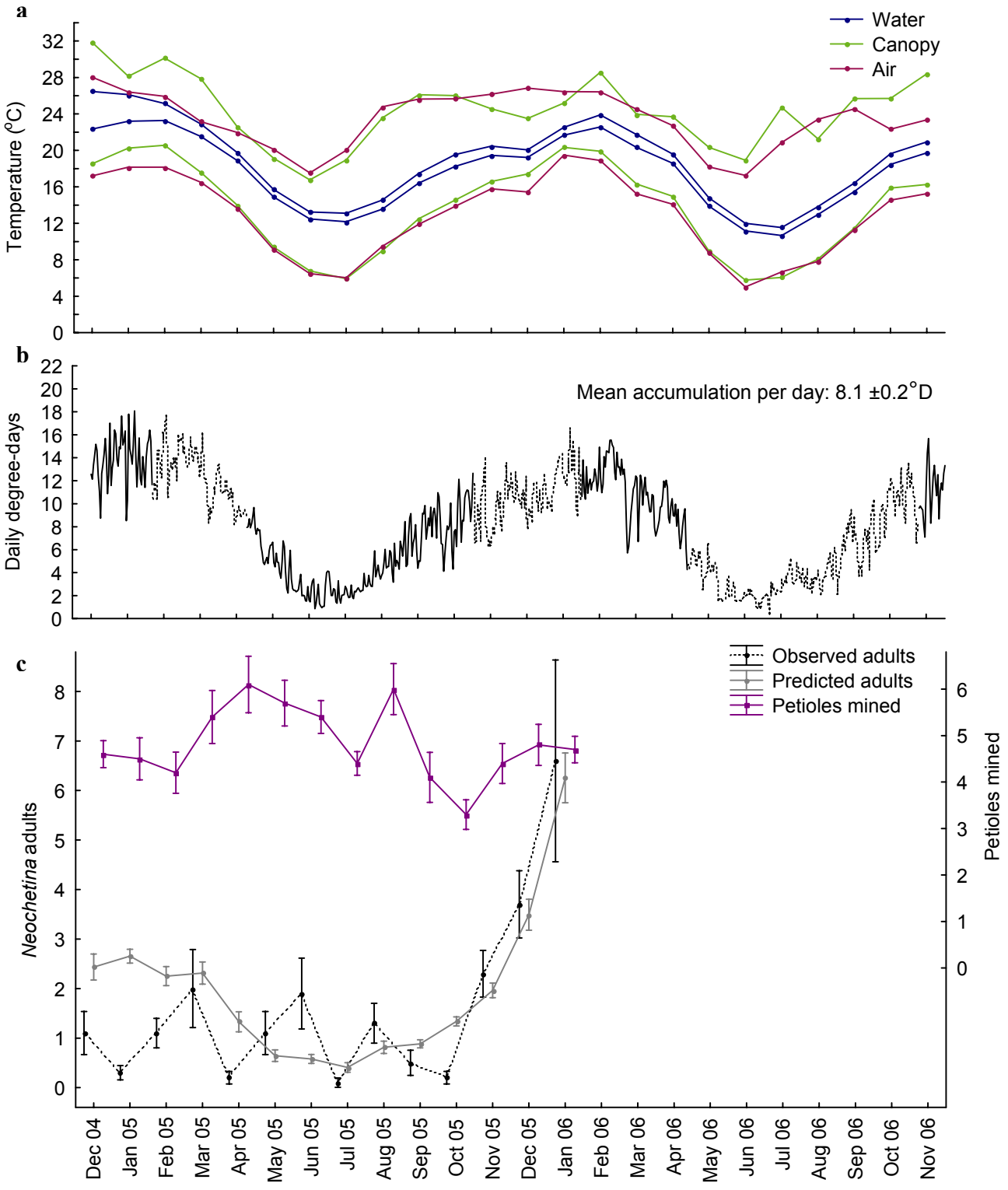


Fig. 3.11. Hammarsdale Dam: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

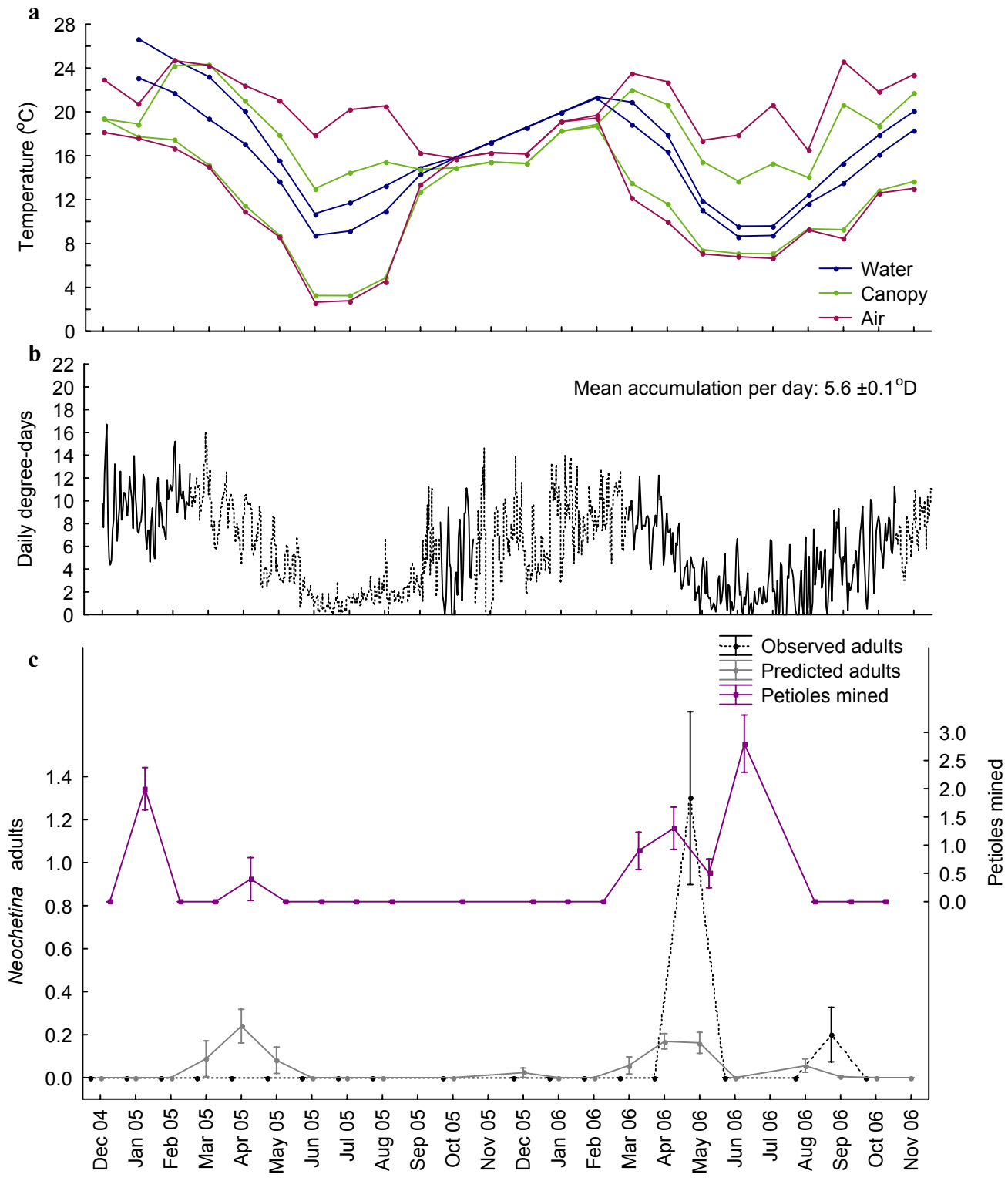


Fig. 3.12. Kubusi River: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

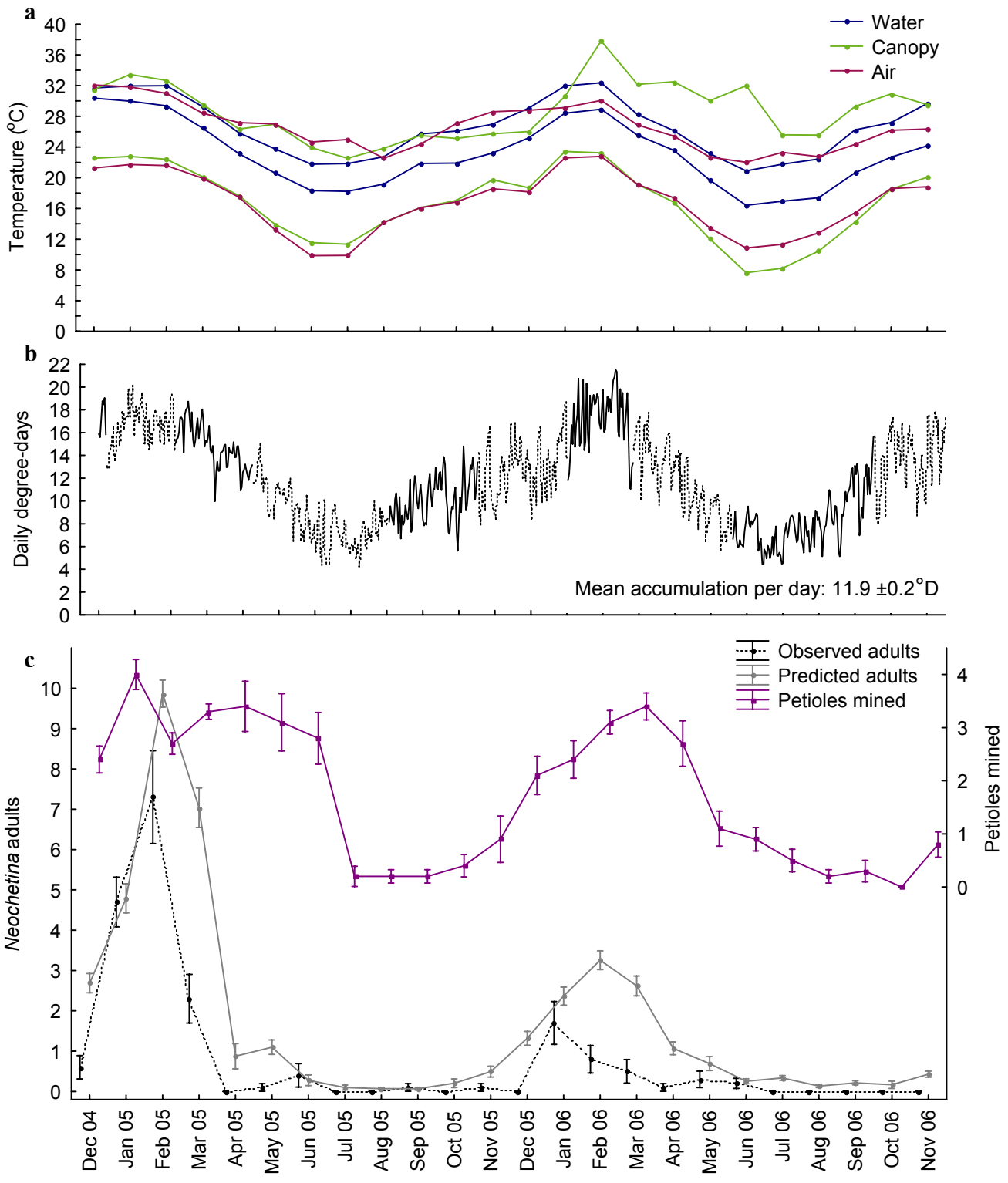


Fig. 3.13. Mbozambo Swamp: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days *(t* = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

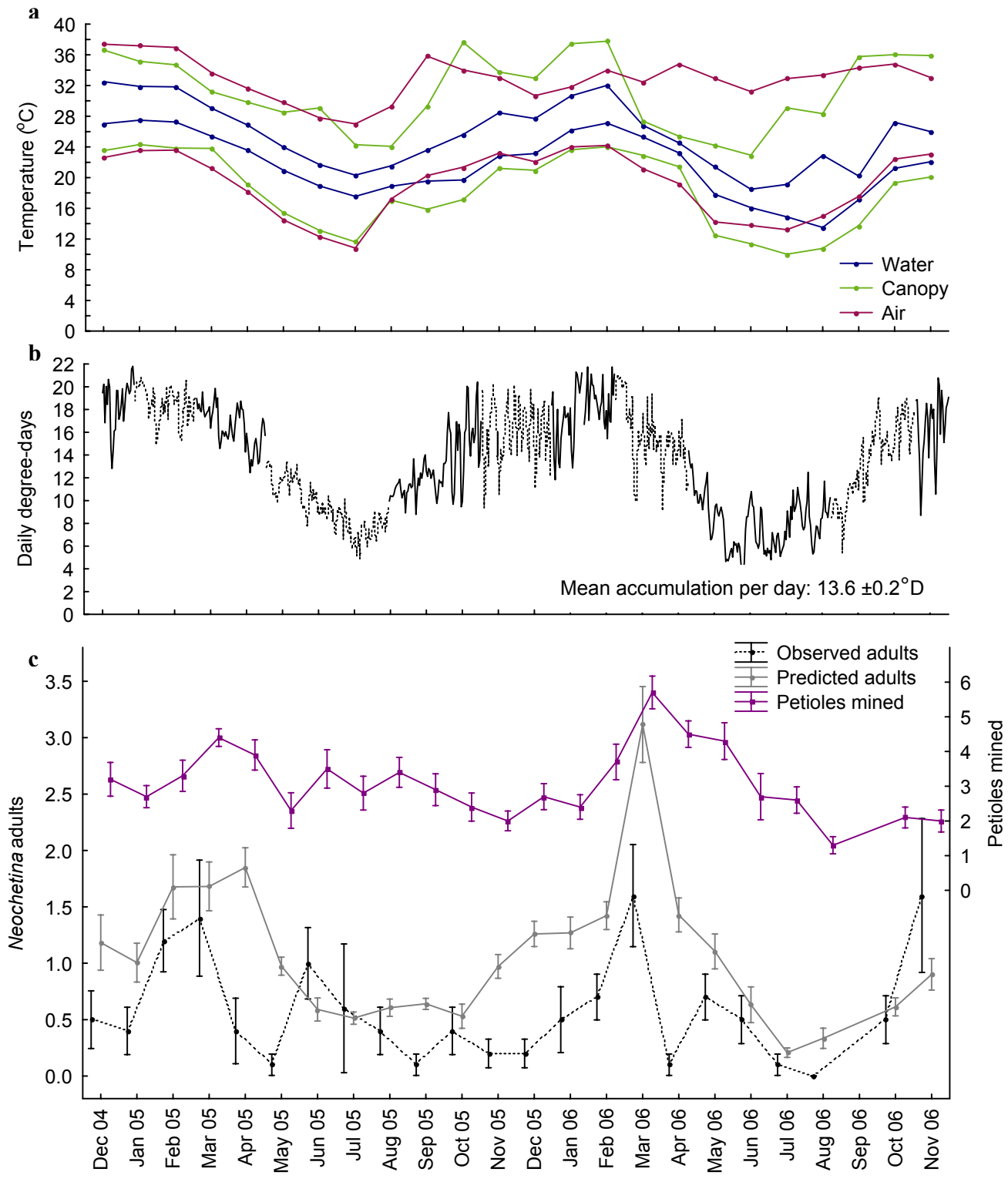


Fig. 3.14. Mkadhzi Spruit: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

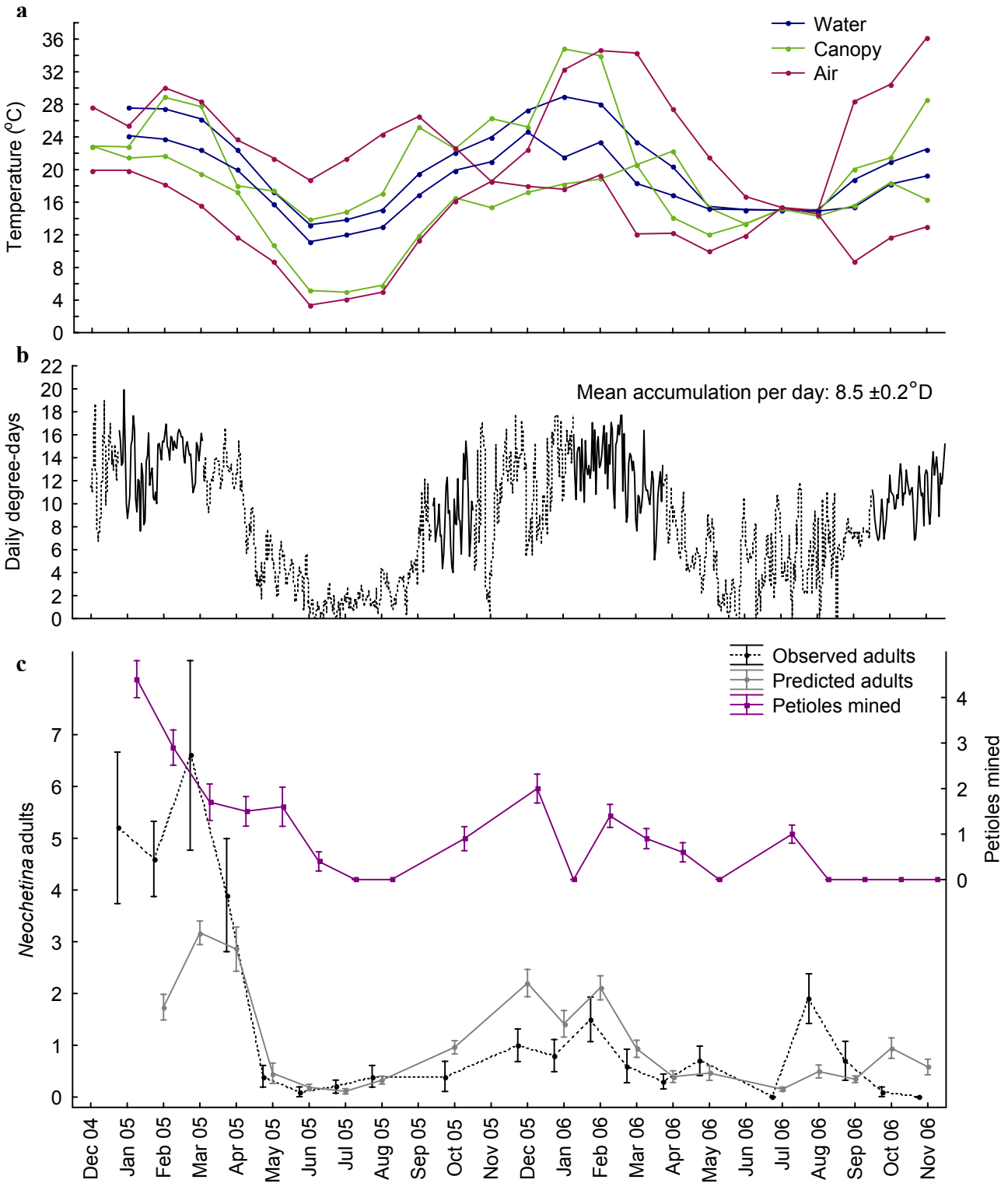


Fig. 3.15. New Years Dam: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days *(t* = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

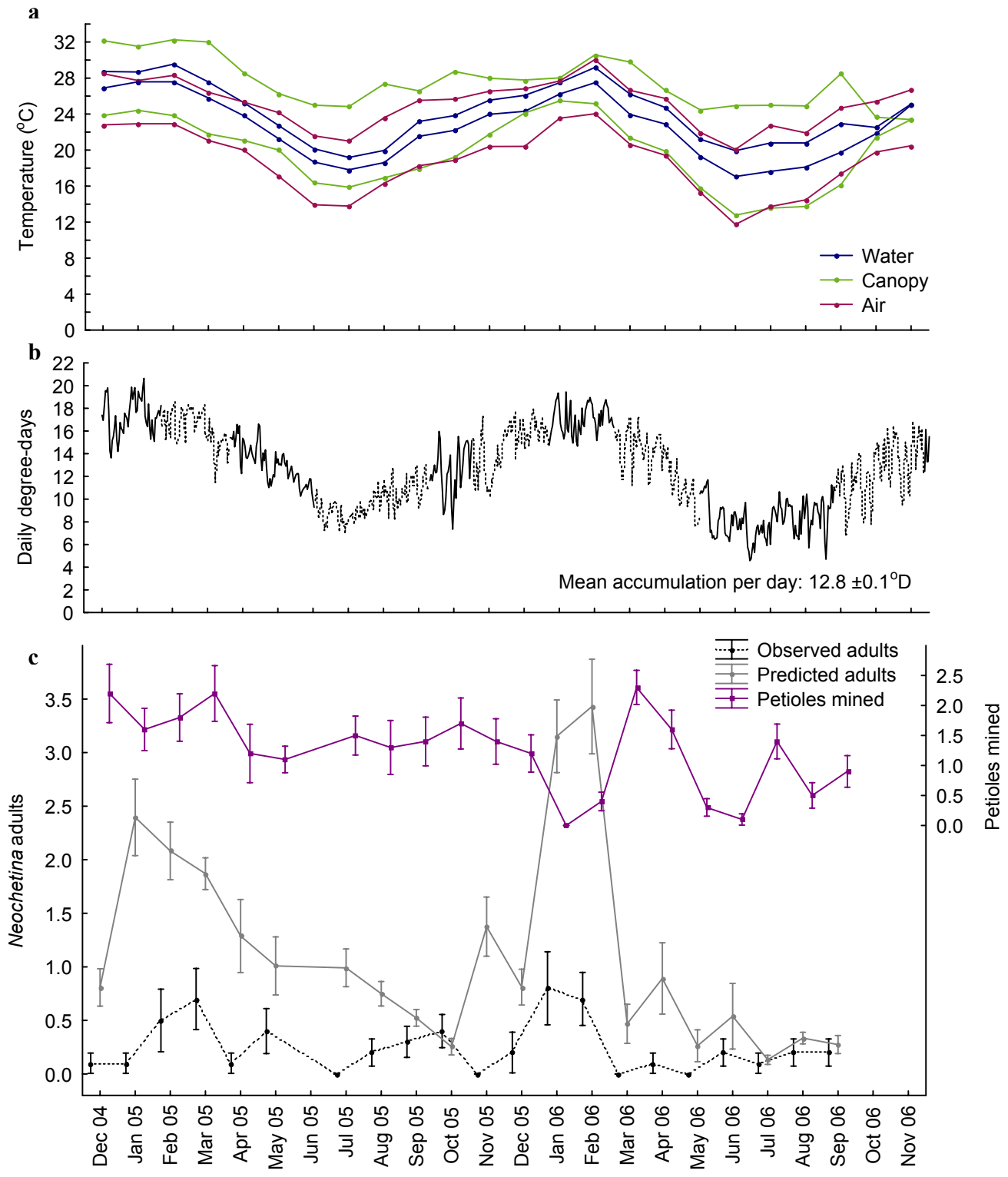


Fig. 3.16. Nseleni River: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

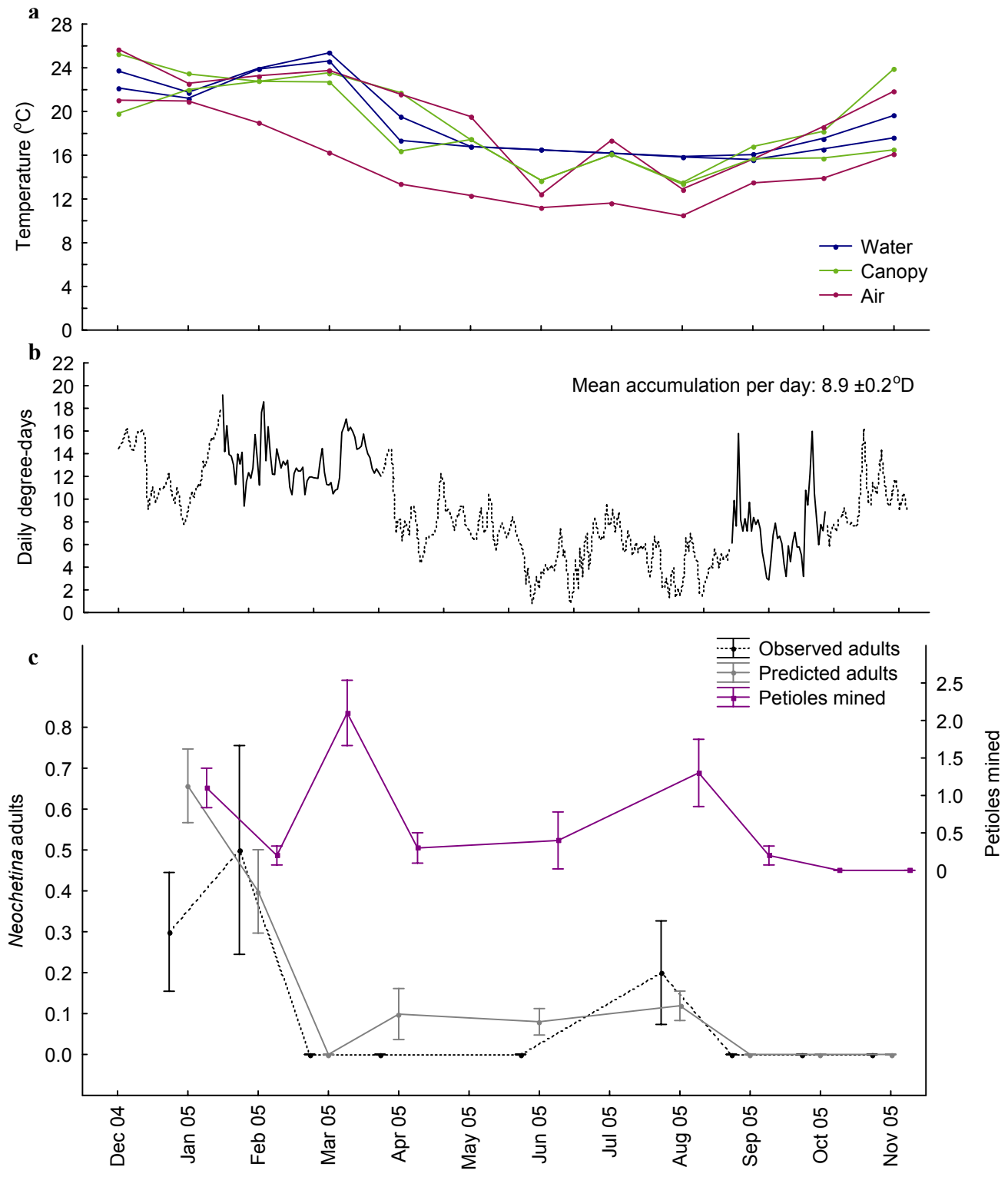


Fig. 3.17. Princess Vlei: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2005. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

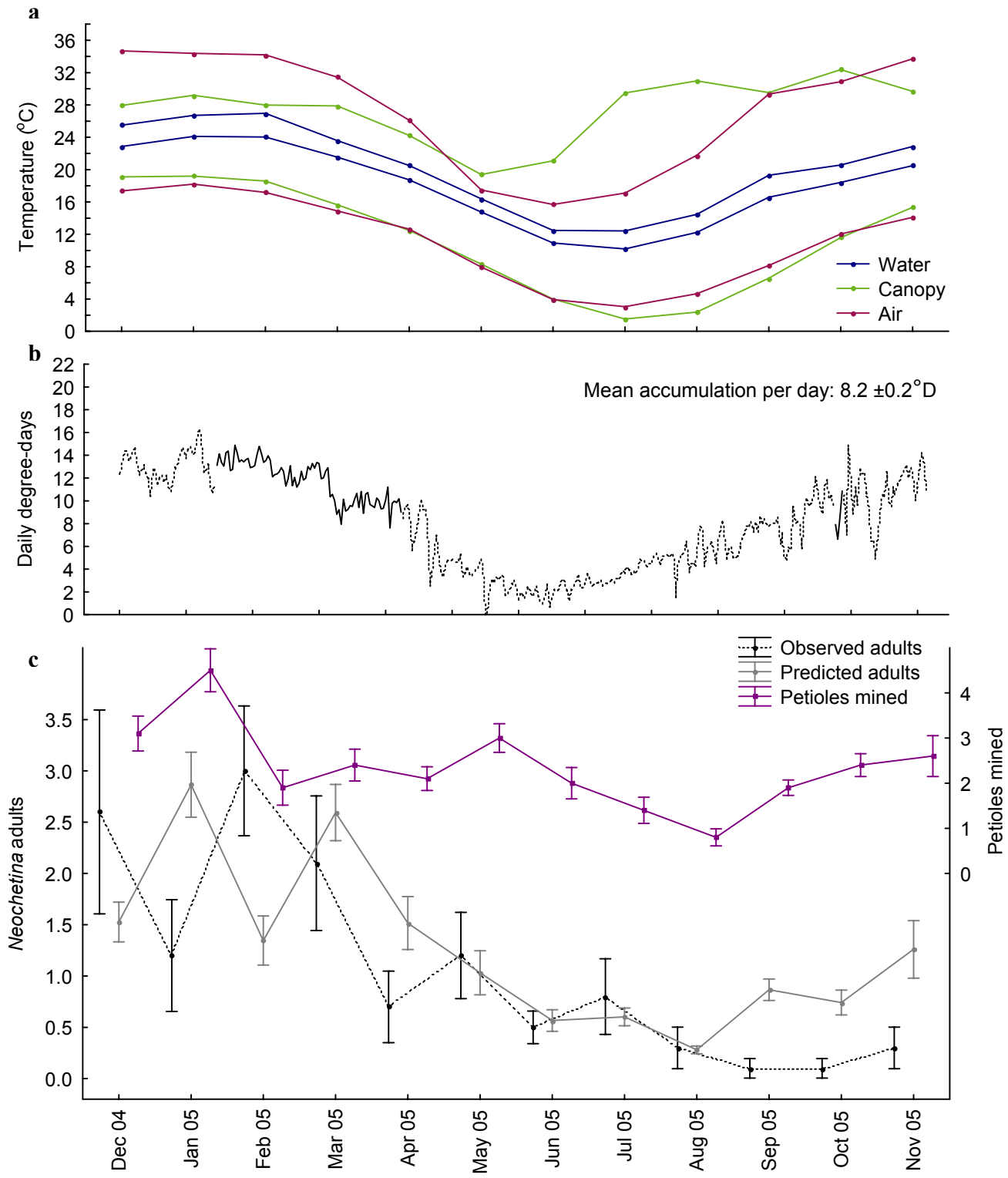


Fig. 3.18. Warrenton Weir: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2005. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

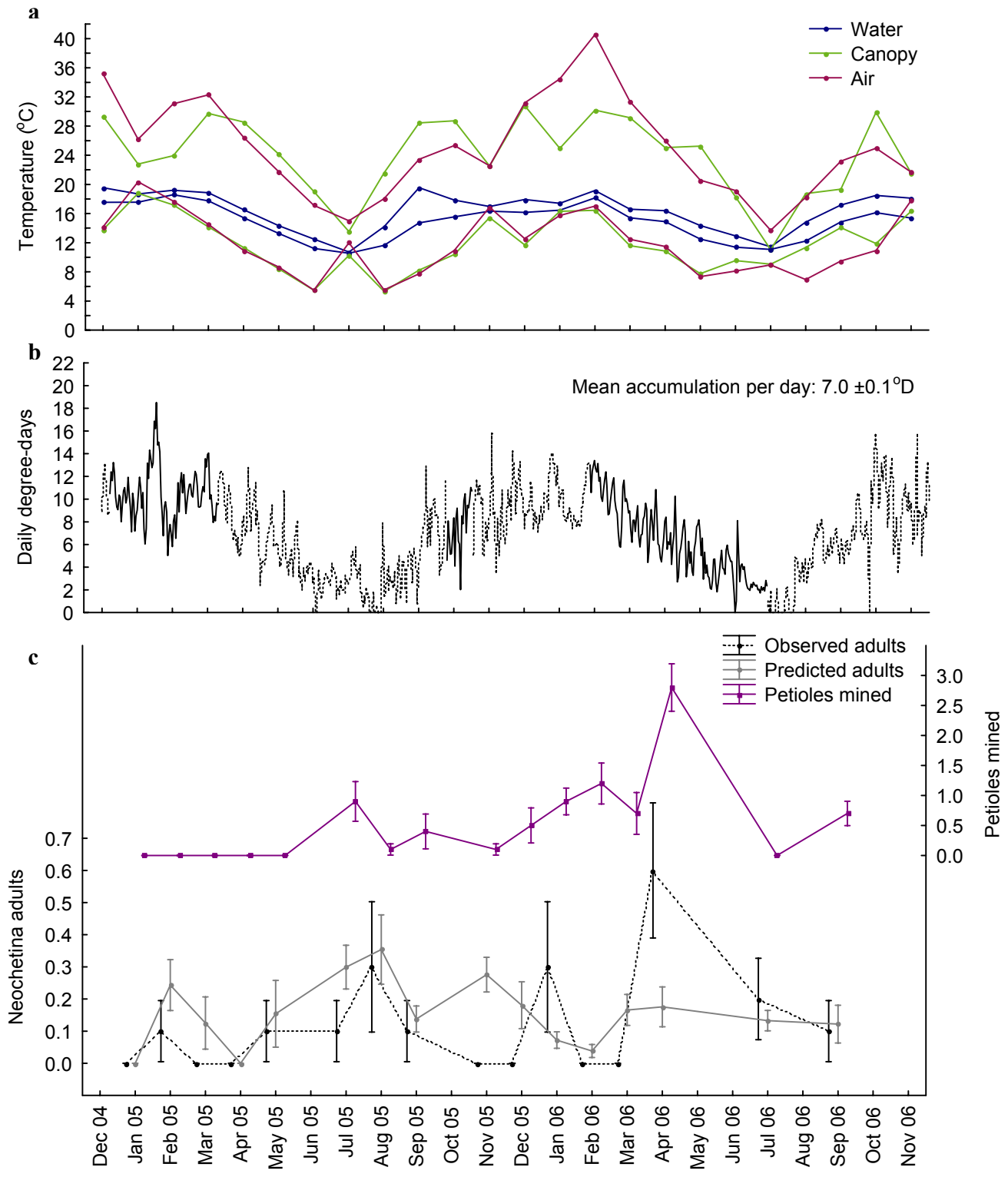


Fig. 3.19. Wolseley: Monthly mean daily maximum and minimum temperature (a) for three microsites; daily degree-days (t = 9.6°C) and predicted *Neochetina* cohorts (K = 984.4°D) starting from 24 October 2005 (b) derived from canopy temperature (different line styles denote cohorts); and *Neochetina* phenology (c) from 2004 to 2006. Weevil phenology is inferred from observed counts of larval mines and adults, and predicted adult numbers are calculated from feeding scars adjusted for monthly leaf production (mean of 10 plants ±SE).

Warrenton Weir, minimum canopy temperatures were buffered whereas during winter they were not (Fig. 3.18a). In all of these cases, maximum petiole length was found to be greater during periods where canopy temperatures were buffered relative to periods not buffered. At Mbozambo Swamp, where buffering of canopy temperatures was evident during winter 2005, maximum petiole length (20.48 ±2.20cm) was significantly greater (t = 2.31; P = 0.02; d.f. = 58) than during winter 2006 (14.44 ±1.41cm). Similarly, at Mkadhzi Spruit (t = 3.91; P < 0.001; d.f. = 185) and Warrenton Weir (t = 13.19; P < 0.001; d.f. = 58), maximum petiole length was found to be significantly greater during times when buffering was observed. Mean maximum petiole length during periods of buffering was 27.60 ±1.22cm and 55.62 ±1.38cm; and 21.64 ±0.94cm and 20.02 ±2.14 during periods where no buffering was evident for Mkadhzi Spruit and Warrenton Weir respectively. Similarly, significant differences in maximum petiole length were also found at Farm Dam and Feesgronde further supporting this relationship. In these cases, canopy temperatures at Farm Dam and Feesgronde were not buffered at mean maximum petiole lengths of 26.26 ±1.27 and 10.93 ±0.72cm. At Delta Park where, no buffering was evident for 19 out of 24 months, mean maximum petiole length oscillated around 14.79 ±0.50cm over the two years of monitoring. Conversely, at Nseleni River where minimum canopy temperatures were buffered relative to air temperatures 20 out of 24 months, mean maximum petiole length over the two years was 38.91 ±1.18cm.

The correlation between plant height and the buffering of lower canopy temperature extremes relative to air temperature lows could not be detected at all sites. This was assumed to be due to the variability across sites caused by non-climatic variables which influence plant population structure and therefore size, such as water nutrient fluctuations and stochastic events like flooding, as well as the plants capacity for vigorous growth. Such rapid changes would be unlikely to be picked up in the relatively coarse monthly monitoring. For this reason only a weak correlation could be found between the two variables across all sites (Fig. 3.20a), evident in the low R^2 value. Nevertheless a significant trend does exist in the data and a better fit was achieved by excluding a number of warmer, less thermally stressful sites (Fig. 3.20b) separated according to the

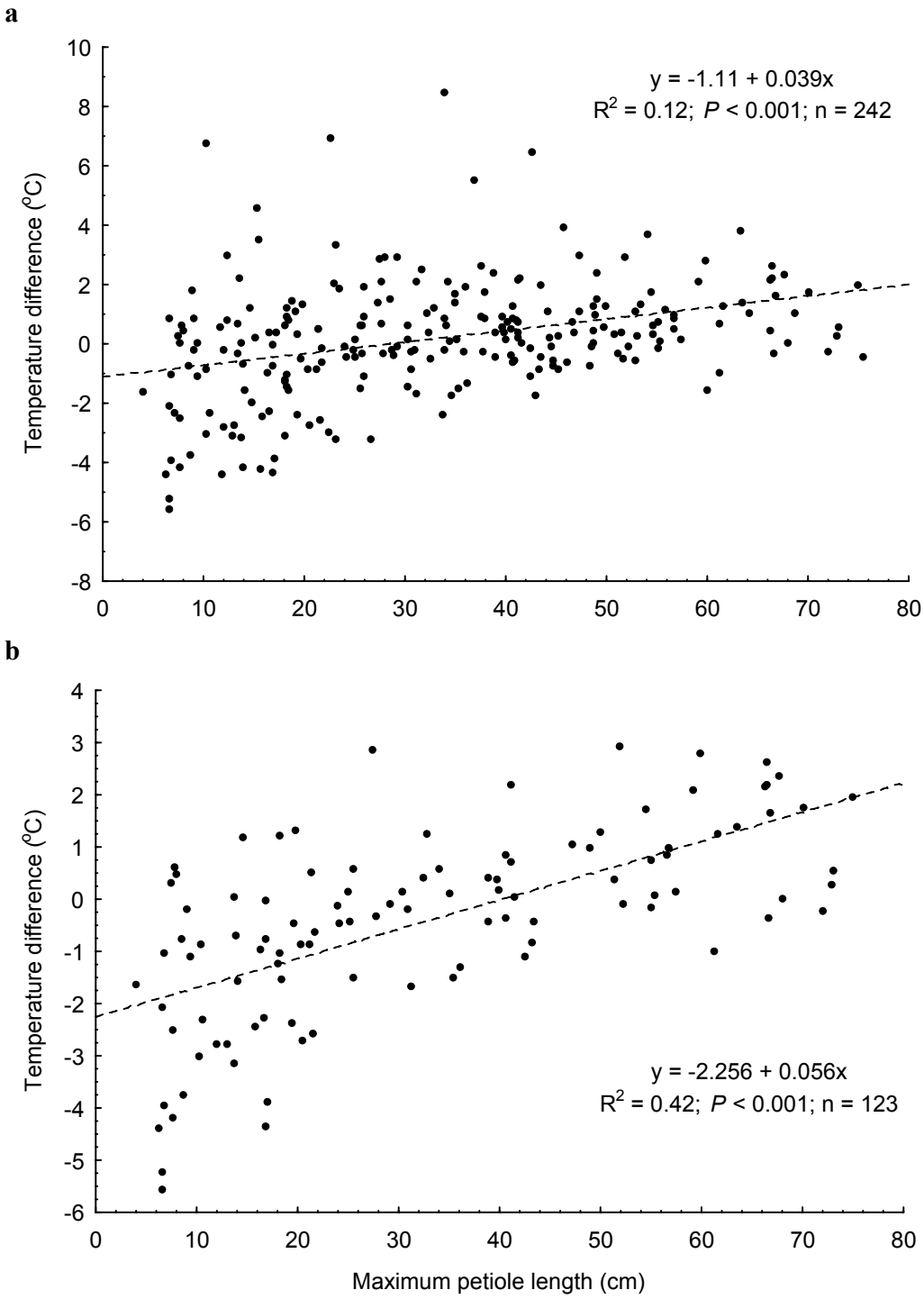


Fig. 3.20. Buffering of lower canopy temperature extremes relative to air temperature lows for all sites (a) and only the relatively colder sites (b) in relation to maximum petiole length (monthly mean from 10 plants). Temperature difference was calculated by subtracting the mean monthly minimum canopy temperature from the corresponding air temperature value.

Principal Components Analysis grouping in Fig. 3.23. The remaining sites are more prone to extremes of temperature and therefore have a greater likelihood of thermal buffering being distinguishable in the data.

Buffering was found to have a significant effect on the accumulation of degree-days. For instance at Mkadhzi Spruit, significantly more degree-days ($t = 5.92$; $P < 0.001$; d.f. = 366) accumulated during the winter of 2005 when buffering of daily minimum temperatures was evident, relative to winter 2006 when daily canopy minima fell below what was recorded in the air temperature profile. On average, up to 1.2 ±0.2 more degree-days per day were recorded during the winter of 2005 despite there being no significant difference ($t = -1.58$; $P = 0.12$; d.f. = 182) between daily maximum canopy temperature during each winter period. Buffering of daily minima, even for this short duration, allowed an extra 109.8 *Neochetina* degree-days to accumulate (Fig. 3.14b), enabling a larger population of weevils to be maintained through the winter of 2005 relative to 2006 (Fig. 3.14c).

3.3.4 *Neochetina* phenology

A high degree of seasonal as well as diurnal variability in the amount of heat available for *Neochetina* development was evident at all field sites (Figs 3.6 - 3.19b). This reflected the variability recorded in the canopy temperature profiles. The amount of heat available for development declined significantly directly preceding, and remained consistently lower, during the winter months at all sites. Consequently, weevil populations at all sites were seasonal with population growth during the favourable summer months followed by a decline or cessation of growth over winter. Using 24 October 2005 as a biofix point, according to the *Neochetina* winter recovery model (Chapter 2: Fig. 2.14), the cooler winter periods translated into prolonged generation times relative to summer, evident from the length or duration of differing line styles in Figs 3.6 to 3.19b. Generation cohorts encompassing winter months were on average 93.07 ±6.74 days longer relative to generations predicted for warmer months. The longest generation time was predicted at Kubusi River lasting 236 days compared with the shortest generation time of 53 days predicted at Mkadhzi Spruit. Periods with no heat available for development, i.e. periods

with a mean canopy temperature below that of the developmental threshold of the weevils, were limited to only six sites and were relatively infrequent, having durations of no longer than six consecutive days. At 15 days per year, Wolseley had the most days on which no degree-days were accumulated. Breede River (9.5 days/year), Crocodile River (4 days/year), Farm Dam (0.5 day/year), New Years Dam (4.5 days/year) and Warrenton Weir (1 day/year) all recorded days on which no degree-days were accumulated. Kubusi River and Delta Park showed the lowest daily mean accumulation of degree-days over the two year monitoring period of 5.6 ±0.1 and 5.9 ±0.1°D/day respectively. Mkadhzi Spruit and Nseleni River were far warmer accumulating more than double this amount on average 13.6 ±0.2 and 12.8 ±0.1°D/day respectively over the two years and were therefore able to consistently sustain far larger insect populations.

Neochetina weevil cohorts were visually difficult to distinguish in the monthly field site data (Figs 3.6 - 3.19c). Peaks in the number of larval mines did not always precede peaks in the adult populations as expected (Fig. 3.21a), nor did the number of field collected or observed adults, and adults predicted from feeding scars, and corrected for leaf turnover, match each other. Averaged over the 14 field sites, predicting the number of adults from feeding scars returned significantly higher population sizes, 0.27 ±0.05 weevils per plant, relative to what was actually found during monitoring (Paired: $t = -5.684$; $P < 0.001$; d.f. = 253). Predicted adult populations were smaller than what was collected at only two sites but these differences were not significant. Predicted adult population numbers correlated better with the occurrence of larval mines, than with observed weevil populations (Fig. 3.21). This correlation was greater at colder sites (grouped according to Fig. 3.23) where distinct generations were more prevalent due to winter synchronising the populations (Fig. 3.21b).

Estimates of the number of generations per year, averaged from counts of recognisable peaks on the plots of larval mines, observed adult numbers, as well as predicted adult numbers (Figs 3.6 - 3.19c) matched the degree-day based predictions of generations per year at 9 of the 14 sites (Table 3.4). The biggest discrepancies between these estimates

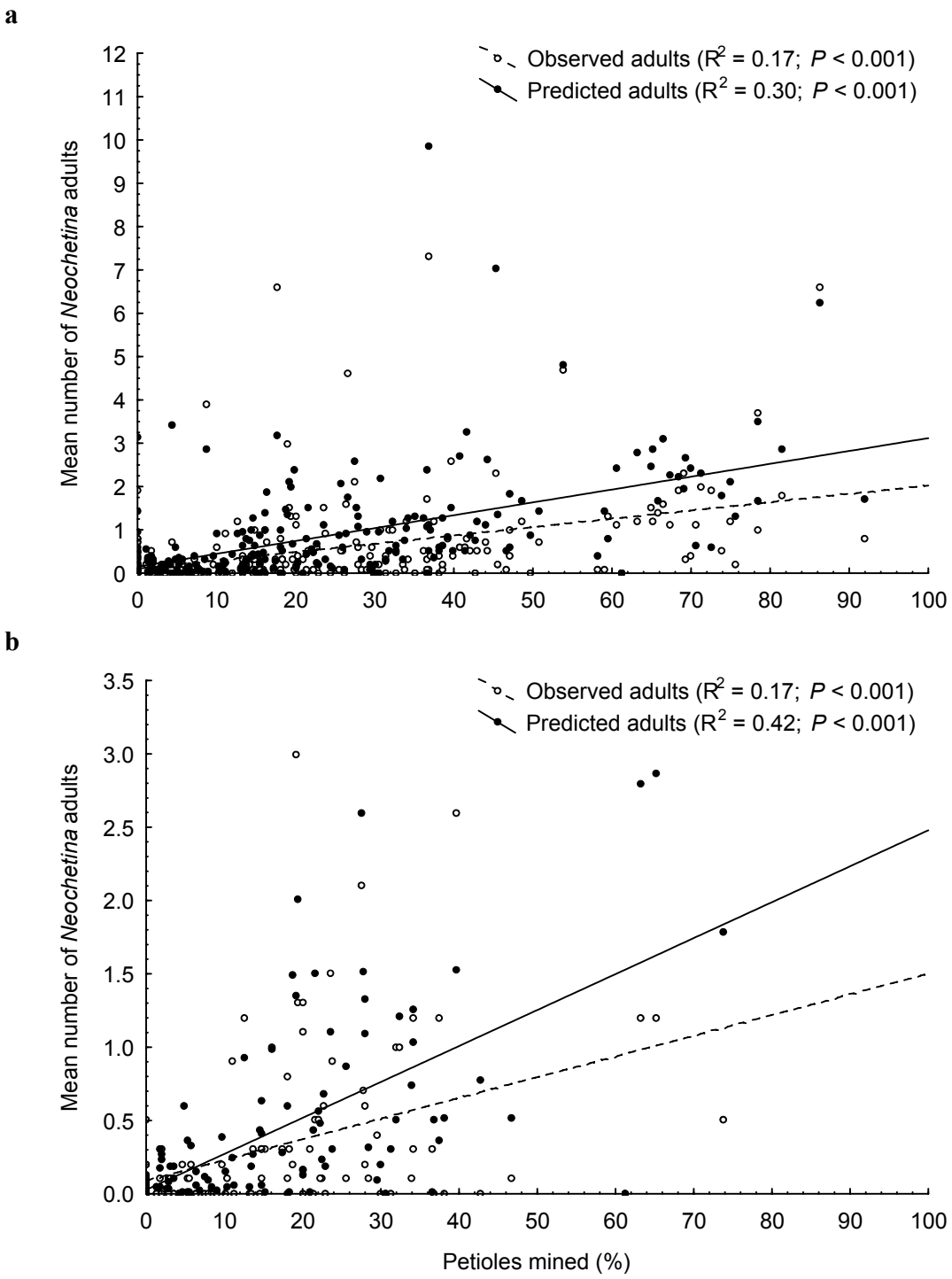


Fig. 3.21. Correlation between the adult *Neochetina* spp. numbers and the occurrence of larval mines at all sites (a) and at only relatively cold sites (b). Points represent monthly means of observed (field collected) and predicted (calculated from monthly feeding scar accumulation) weevil adults.

occurred at Mbozambo Swamp, Mkadhzi Spruit and Nseleni River, where *Neochetina* population dynamics were less stochastic and, due to year-round favourable temperature, lacked distinct cohorts or generations. This led to counts of fewer generations than expected at these sites relative to the predictions based on the accumulation of degree-days. Timing of peaks in larval mines, observed and predicted adult numbers (Figs 3.6 - 3.19c) were difficult to match to changeover points between cohorts determined by degree-days. Nevertheless, the number of generations predicted via either method was not significantly different (Paired: $t = 1.77$; $P = 0.10$; d.f. = 13).

Disturbances had a profound influence on *Neochetina* populations at a number of sites throughout the field sampling. Flooding at Breede River caused the premature cessation of monitoring as the majority of plants were washed out of the system (Fig. 3.6c). Similarly, at Crocodile River at the beginning of January 2006, a flood reduced the water

Table 3.4. Comparison of the predicted number of *Neochetina* weevil generations per year calculated from degree-day accumulation from the canopy temperature profile, with an estimate of generations averaged from counts of recognisable peaks in larval mines and adult weevils per year (Figs. 3.6 - 3.19c).

Site Name	Predicted number of generations ($year^{-1}$ ±error)	Estimated number of generations (peaks/year ±SE)
Breede River	2.78 ±0.03	2.00 ±0.57
Crocodile River	3.09 ±0.06	4.30 ±0.33
Delta Park	2.19	2.80 ±0.31
Farm Dam	2.36	2.80 ±0.31
Feesgronde	3.22 ±0.007	2.00 ±0.57
Hammarsdale Dam	3.02 ±0.01	3.00 ±0.57
Kubusi River	2.08 ±0.22	1.67 ±0.33
Mbozambo Swamp	4.39 $\pm 9^{-4}$	2.00
Mkadhzi Spruit	5.03 ±0.005	2.83 ±0.48
New Years Dam	3.16 ±0.05	2.83 ±0.31
Nseleni River	4.74 ±0.03	3.17 ±0.40
Princess Vlei	3.32 ±0.35	2.67 ±0.33
Warrenton Weir	3.03 ±0.002	3.67 ±0.33
Wolseley	2.59 ±0.01	2.17 ±0.31

hyacinth population from a mat of roughly 700m^2 down to a fringing population of less than 8m^2. The loss of plant material resulted in a steady decline in the population of the weevil, and consequently numbers were reduced to negligible levels where they remained for the next seven months (Fig. 3.7c). Conversely, an influx of nutrients caused by a sewage spill into Farm Dam towards the end of October 2005 increased plant vigour leading to greater weevil population numbers during the following summer relative to the same time period the year before (Fig. 3.9c).

At Feesgronde, flooding in mid February 2005 washed out most plants at the site (Fig. 3.10c). As a result, the resident weevil population declined dramatically and, due to a number of frequent flushes repeatedly removing plants from the site, were slow to recover. Throughout sampling at Hammarsdale Dam, water hyacinth at the site, a narrow inlet to the dam roughly 15m wide, was gradually out competed by wetland plants (*Ludwigia* sp. (Onagraceae)) and terrestrial grasses. As the water hyacinth mats receded, weevil populations became increasingly concentrated on what little plant material remained leading to abnormally high population spikes in January 2006 before crashing (Fig. 3.11c). Repeated flooding was also problematic at Kubusi River where continual removal of plant material disrupted weevil populations and prevented any significant population build up (Fig. 3.12c). Mbozambo Swamp was subjected to a herbicide spray in December 2004 causing widespread plant mortality. *Neochetina* populations gradually became concentrated on plants that were missed by the herbicide application leading to abnormally high population peaks in February 2005 before crashing. Plants were slow to recover, possibly due to increased wind and wave action brought on by the increased areas of open water, and consequently weevil numbers were only seen to resurge in 2006 during the following growth season (Fig. 3.13c).

3.3.5 *Eccritotarsus catarinensis* phenology

Large mirid populations were only recorded at four of the 14 field sites, Breede River, Crocodile River, Hammarsdale Dam and Kubusi River. Mirid generations or cohorts could not be isolated within the data, instead mirid activity fluctuated in response to seasonal temperature change, with greater population numbers evident in the more

favourable summer months (Fig. 3.22a-d). Nevertheless, in some cases populations appeared to undergo distinct 'boom and bust' cycles independent of the broader seasonal trends (Fig. 3.22a, c and d). These cycles could not be successfully correlated to any particular abiotic variable, although in some instances, 'bust' periods coincided with small declines (on average 2.3kg/m^2) in plant biomass.

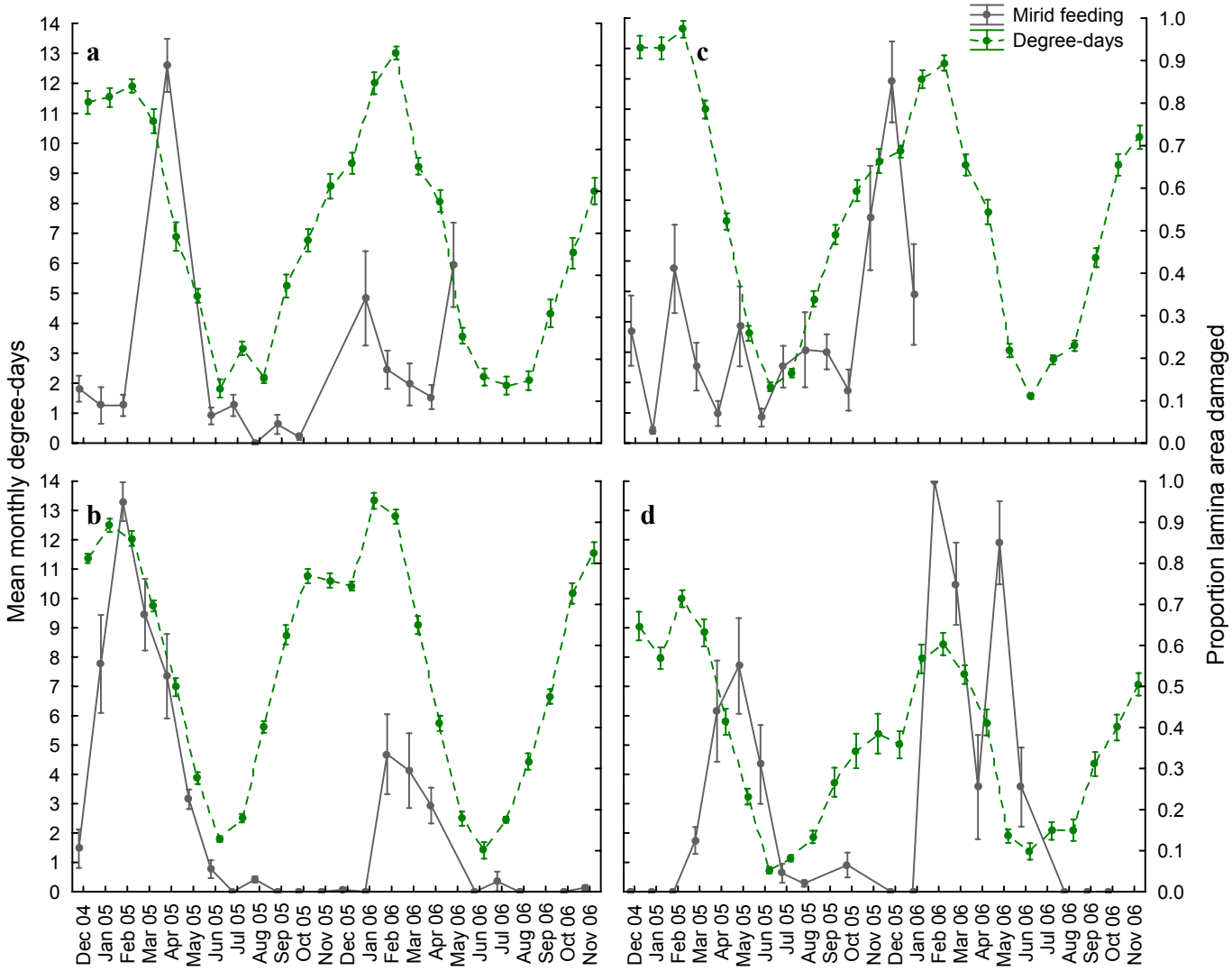


Fig. 3.22. *Eccritotarsus catarinensis* feeding intensity relative to the mean monthly degree-days (calculated from degree-hours) recorded from 2004 to 2006 at four field sites, Breede River (a), Crocodile River (b), Hammarsdale Dam (c), and Kubusi River (d). Feeding damage was ranked between 0 (no leaf area damaged) and 1 (50 to 100% leaf area damaged) (mean from 10 plants ±SE).

3.3.6 Stress indices

Degree-day modelling indicated how broad climatic trends limit natural enemy activity by slowing development and generation turnover at colder sites. Nevertheless, more acute temperature based factors linked directly to an organism's physiology enabled a better understanding of the variability evident between locations. Principal Components Analysis allowed for separation of the field sites into distinct groups based on these acute factors or the amount of climatic 'stress' experienced by each organism at a given site.

Whilst Principal Components Analyses grouped sites similarly for both *E. catarinensis* and the *Neochetina* weevils, when analysed separately, the relative contribution of each stress index in explaining the variance between sites differed for each species. When differentiating between sites based on the perceived stressors on the *Neochetina* weevils (Table 3.5), 88.9% of the variation among sites could be explained by the principal component or largest eigenvalue produced by the analysis. The relative contribution of each of the four eigenvectors or stress indices to this component was roughly similar with the feeding index (26.1%) making the highest, and oviposition index (23.3%) the lowest contribution. This suggests that *Neochetina* efficacy and possibly distribution are limited primarily by the number of cold days per annum with a mean nocturnal temperature of less than 6.3°C. Although the second factor accounted for only 8.0% of the variance between sites, it was strongly inversely dominated by the oviposition index. Thus, the relative importance of the oviposition threshold declined with decreasing temperature making differentiation with this index only possible between the warmer sites.

When differentiating between sites based on perceived *E. catarinensis* stress (Table 3.6), the analysis was again dominated by a single eigenvalue accounting for 81.0% of the variation. The variability inherent in this principal component, as was the case in the *Neochetina* analysis, was evenly spread between the four stress indices. The maintenance index was the most prominent eigenvector explaining 26.7% of the variation in the principal component while the feeding index (22.8%) was the least significant. Differentiating between sites based on the number of consecutive cold days as described

Table 3.5. Climatic stress indices for the *Neochetina* weevils at 14 field sites distributed around South Africa. (OvI: Mean nocturnal hours < 12.5ºC per day per year; MaI: Max consecutive days with mean < 9.6ºC pa; FeI: Days with nocturnal mean < 6.3ºC pa; MoI: Max consecutive days with daily minimum < 3.8ºC pa).

Site Name	Oviposition index [OvI] (Hrs/day/year)	Maintenance index [MaI] (Days/year)	Feeding Index [FeI] (Days/year)	Mortality index [MoI] (Days/year)
Breede River	2.3	4.0	2.0	0.5
Crocodile River	3.5	12.5	45.5	8.0
Delta Park	5.4	51.5	107.5	77.0
Farm Dam	4.8	55.5	94.0	44.5
Feesgronde	4.0	21.0	60.0	34.0
Hammarsdale Dam	3.0	6.5	12.0	2.5
Kubusi River	3.9	9.0	32.0	9.5
Mbozambo Swamp	1.1	0.0	1.5	1.5
Mkadhzi Spruit	0.7	0.0	0.0	0.0
New Years Dam	2.3	4.0	9.5	3.0
Nseleni River	0.1	0.0	0.0	0.0
Princess Vlei	0.7	0.0	0.0	0.0
Warrenton Weir	4.4	8.0	62.0	22.0
Wolseley	4.0	5.0	12.5	4.0

Table 3.6. Climatic stress indices for *Eccritotarsus catarinensis* at 14 field sites distributed around South Africa. (OvI: Mean diurnal hours < 10.8ºC per day; MaI: Max consecutive days with mean < 10.3ºC pa; FeI: Days with diurnal mean < 10.8ºC pa; MoI: Max consecutive days with daily minimum < 1.2ºC pa).

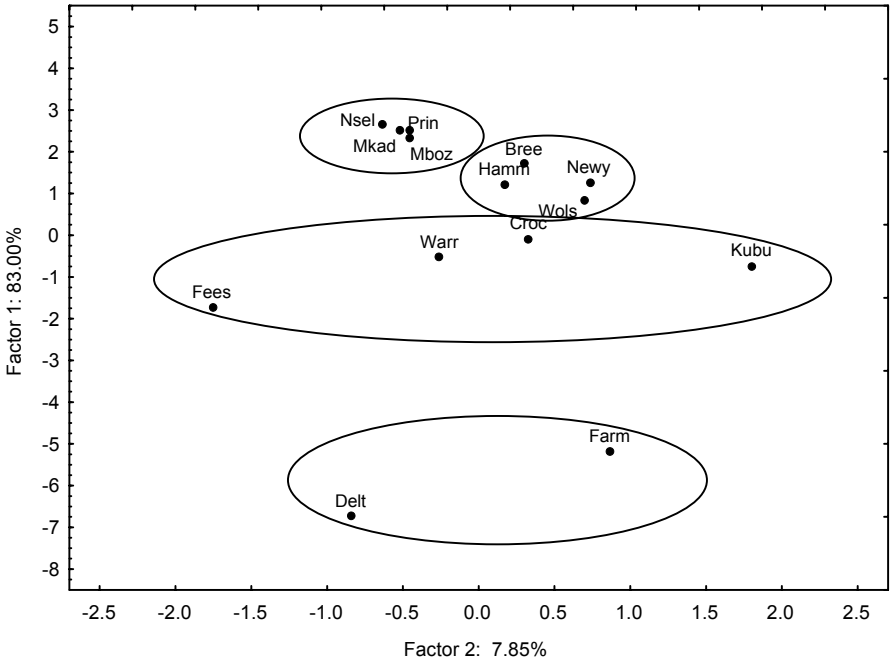
Site Name	Oviposition index [OvI] (Hrs/day/year)	Maintenance index [MaI] (Days/year)	Feeding Index [FeI] (Days/year)	Mortality index [MoI] (Days/year)
Breede River	1.2	4.5	17.0	0.0
Crocodile River	3.0	20.5	19.0	3.0
Delta Park	4.8	68.50	50.0	20.0
Farm Dam	4.4	72.50	64.5	15.0
Feesgronde	3.5	22.0	3.0	14.0
Hammarsdale Dam	4.4	7.5	7.5	0.0
Kubusi River	3.0	14.0	57.0	2.5
Mbozambo Swamp	0.7	0.0	0.0	0.0
Mkadhzi Spruit	0.4	0.0	0.0	0.0
New Years Dam	1.6	6.5	33.5	1.0
Nseleni River	0.02	0.0	0.0	0.0
Princess Vlei	0.1	0.0	3.0	0.0
Warrenton Weir	3.7	8.0	14.0	4.0
Wolseley	2.6	7.0	21.5	0.5

by the maintenance index is especially important for short lived species such as *E. catarinensis*.

As the effects of cold on water hyacinth were seen to negatively affect both the weevils and *E. catarinensis*, field sites were also differentiated by incorporating factors affecting plant growth and quality (Table 3.7). Considering the stress indices from all three species together, the Principal Components Analysis (Fig. 3.23) was again dominated by a single factor accounting for 83.0% of the variation among the sites. No single eigenvector or stress index dominated the principle component but the *Neochetina* feeding (10.7%) and *E. catarinensis* maintenance (10.6%) indices were again prominent. Only five eigenvectors explained more than 10.0% of the variation between sites as described by the principal component. These indicated that consecutive days with consistently low mean temperatures (maintenance indices), cold nocturnal temperatures (*Neochetina* feeding index), and extreme temperature minima (mortality indices) were most useful for differentiating between field sites in terms of suitability for the insects. As the dominant

Table 3.7. Climatic stress indices for *Eichhornia crassipes* at 14 field sites around South Africa. (GrI: Days with mean water temperature < 10°C pa; FrI: Frost days per year).

Site Name	Growth index [GrI] (Days/year)	Frost index [FrI] (Days/year)
Breede River	0.0	0.0
Crocodile River	12.0	3.0
Delta Park	99.0	46.5
Farm Dam	78.0	19.5
Feesgronde	0.0	26.0
Hammarsdale Dam	4.0	0.0
Kubusi River	48.0	5.5
Mbozambo Swamp	0.0	0.0
Mkadhzi Spruit	0.0	0.0
New Years Dam	0.0	0.5
Nseleni River	0.0	0.0
Princess Vlei	0.0	0.0
Warrenton Weir	0.0	11.0
Wolseley	0.5	0.0



Eigenvectors (% contribution)		
Variable	**Factor 1** (83.00%)	**Factor 2** (7.85%)
***Neo* Ovl**	9.35	9.39
***Neo* Mal**	10.61	1.00
***Neo* Fel**	10.65	4.15
***Neo* Mol**	10.54	11.45
***Ecc* Ovl**	9.89	5.66
***Ecc* Mal**	10.56	1.52
***Ecc* Fel**	8.24	27.97
***Ecc* Mol**	10.39	13.12
***Eic* Grl**	9.81	9.01
***Eic* Frl**	9.96	16.74

Fig. 3.23. Grouping of field sites using principle components analysis incorporating 10 thermal stress indices determined for the *Neochetina* weevils (*Neo*), *Eccritotarsus catarinensis (Ecc)* and *Eichhornia crassipes (Eic)*.

factor in the analysis accounted for 83.0% of the variation, sites were grouped primarily by differences along the y-axis of Fig. 3.23. Four distinct groups were isolated, the most inhospitable comprised Delta Park and Farm Dam and the least stressful consisted of Mbozambo Swamp, Mkadhzi Spruit, Nseleni River and Princess Vlei. Ranking sites from coldest to warmest according to the yearly accumulation of degree-days (Fig. 3.24) indicated that the consistently colder sites in some cases were not necessarily the most stressful. Although found to be the coldest site, Kubusi River was not ranked with the most thermally stressful sites Delta Park and Farm Dam. Other sites, similarly located in the interior regions of the country, but at slightly lower altitudes, for example, Crocodile River, Feesgronde and Warrenton Weir were also found to be less stressful than Delta Park and Farm Dam.

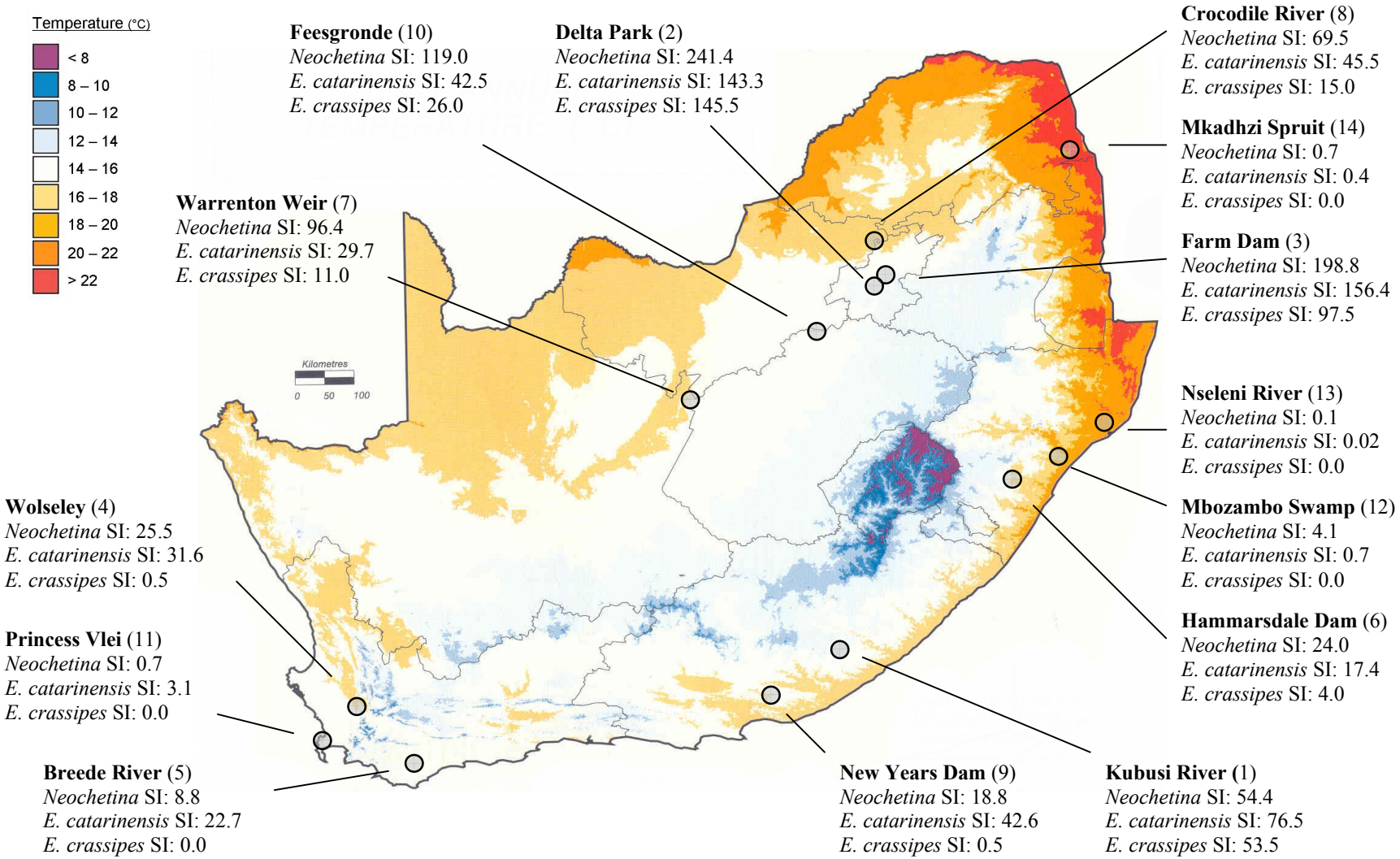


Fig. 3.24. Relative stress indices for 14 climatically distinct water hyacinth infested field sites based on physiologically relevant thresholds for the *Neochetina* weevils, *Eccritotarsus catarinensis* and *Eichhornia crassipes*. Sites are ranked from coldest to warmest according to the yearly accumulation of degree-days. Map depicts the mean annual temperature for South Africa ranging (Source: Schulze *et al*. 1997).

3.4 Discussion

3.4.1 Data handling

Despite the assumptions behind the construction and use of degree-day based predictions of insect and plant development, the approach is widely used due to its simplicity and practical value. Nevertheless, Herms (2004) stipulates that the greatest source of error in degree-day models lies in the temperature data used to calculate degree-days. In order to be accurate, temperature based models must be constructed from data that are representative of the organism's habitat, as well as being as homogenous as possible. Aguilar *et al*. (2003) defines a homogenous climatic time series as one where variation within the data is caused only by variations in climate. Unfortunately, high resolution or long-term climatic data sets are seldom free of inhomogeneities and this study is no exception. Inhomogeneities make data unrepresentative and can bias datasets leading to misinterpretations. Aguilar *et al*. (2003) stress the importance of removing inhomogeneities altogether or at least to determine the possible error they may cause.

The temperature data or time series collected during this study were affected by two main inhomogeneities, namely radiative heating errors and missing sequences of data of varying degrees and durations. Radiative heating errors in both the air and canopy temperature profiles led to inflated temperature readings and therefore an overestimation of accumulating degree-days. Missing sequences of data were evident in temperature profiles from all three microsites and depending on the duration, had the potential to influence seasonal trends or omit stochastic events such as heat waves or cold fronts making predictions unreliable.

In a study on possible environmental applications of Thermochron iButtons, Hubbart *et al*. (2005) investigated the potential for radiative heating under worst case conditions, namely exposure to solar radiation with little to no ventilation. iButtons were placed into both aspirated and non-aspirated six-plate radiation shields as well as left unshielded in greenhouse conditions. A maximum temperature difference of 16.5°C was observed between the aspirated radiation shield and the unshielded iButton which the authors suggest is excessively high due to the low natural ventilation within the greenhouse

setting. Nevertheless, this value is small compared to the radiative heating errors recorded during this study within some of the air temperature datasets. The greatest difference between a time series subjected to radiative heating and the offset weather station data was recorded at Wolseley with daily maximum temperatures on average 26.6°C higher, although other radiative heating errors did not approach this magnitude.

Using the water temperature profile as a reference time series for correcting radiative heating errors provided a conservative estimate of 'real' temperatures in that this method preserved the high resolution of the hourly dataset. Additionally, it is difficult to quantify the relative extent of radiative heating between months and sites under varying circumstance, and thus, water temperature provided a consistent site and time specific reference dataset. However, as was the case in Fig. 3.4a, some time series remained significantly higher than the corresponding offset weather station data even after adjustment. In terms of biological significance however, over inflation of the air temperature profile at any given site was relatively small and would account for only a small proportion of a *Neochetina* generation. At New Years Dam, due to the long duration (92 days) of the adjusted period, significantly higher air temperature values would only account for 20.00% of a single *Neochetina* generation over the year. The most inflated values, recorded at Wolseley, had a similarly small influence on predicted *Neochetina* generational turnover amounting to only 5.63% of a single generation per year.

Radiative heating of the canopy temperature probe was far more common and prevalent throughout all of the field sites. Adjusted canopy temperature profiles were prone to the same problems found when adjusting air temperature errors. Whilst the majority of data series adjusted did not differ significantly from the offset weather service data in terms of daily means, some series remained significantly warmer and some cooler following adjustment. Additionally, because data series were adjusted according to monthly measures of petiole length, and changes in petiole length occur gradually and are not restricted by calendar dates, it is inevitable that some sequences of data were adjusted unnecessarily and conversely, other sequences that needed to be adjusted were not.

Radiative heating could also arise if canopy probes became exposed by unforeseen plant movements due to wind and wave action that would have been overlooked if petiole lengths were longer than the prescribed 15cm. These exposure and transition periods however, were unlikely to exceed a month and must be expected to balance each other out yielding approximately correct values and consequently good estimates of accumulating degree-days. In the absence of a complete minute-by-minute record of sensor exposure, this method provided an empirical and unbiased way of correcting radiative heating errors.

A variety of complex analytical techniques exists for predicting and filling missing temperature values omitted from temperature datasets (Mraoua and Bari, 2007; Kotsiantis *et al*. 2006). In most cases however, these techniques are restricted to long duration time series (>10 years) and not to finer-scale datasets where simpler techniques can often out-perform more complex ones (Mraoua and Bari, 2007). Homogenisation of the temperature data in this study using the modified fallback approach provided a suitably accurate method for filling gaps in that its associated error lay within biologically relevant boundaries. Using offset reference time series from nearby weather stations also allowed seasonal trends over longer gaps, as well as the majority of stochastic events, to be preserved in the datasets that might otherwise have been omitted using simpler techniques such as linear trends to fill gaps.

One drawback of solely using the modified fallback approach is the exclusion of microsite-specific diurnal trends from which to determine degree-days. Using hourly temperature data offers the greatest accuracy when calculating degree-days (Cesaraccio *et al*. 2001; Roltsch *et al*.1999). Despite this, the datasets used in the reference time series employed throughout this study were constructed from offset weather station data consisting of only daily maximum and minimum temperatures. Daily mean temperatures calculated from these two values were used for all subsequent degree-day accumulations for 'missing-data' periods despite the existence of numerous models able to approximate diurnal temperature trends from daily maximum and minimum temperature values (Roltsch *et al*. 1999; Zalom *et al*. 1983). Nevertheless, much debate exists on which

technique is best and some studies have even shown that there is little advantage to using data which incorporate the diurnal cycle. In a study on the thermal requirements of warm-season crops, Purcell (2003) found that there was no advantage in using hourly temperature data over daily values as both methods produced similar predictions. Accumulating degree-days for all 'missing data' periods from means which incorporate the diurnal cycle will undoubtedly be most accurate but most likely unnecessary given the already negligible error associated with the predicted number of insect generations at each site (Tables 3.2 and 3.3).

Adequate data homogenisation is confirmed by the good agreement between the number of *Neochetina* generations at each site predicted by degree-day accumulation, and the inferred number of generations estimated from adult numbers derived from observed counts and those predicted from feeding scars (Table 3.4). Newman *et al*. (2001) found that the weevil *Euhrychiopsis lecontei* Dietz (Curculionidae), a biological control agent of Eurasian water milfoil which is also prone to long periods of inactivity associated with overwintering, was capable of similar generational turnover, producing three to six generations per year in North America. In Argentina, DeLoach and Cordo (1976a, b) found that *Neochetina bruchi* could complete between three and four generations per year. Similarly, Stark and Goyer (1983) found that in southern Louisiana with a mean winter temperature of 14°C, *Neochetina eichhorniae* was capable of completing up to four generations per year. Given this comparable generational turnover, as well as the good correlation with other field measures, for example the number of larval mines, aspects of *Neochetina* and *E. catarinensis* phenology derived from the homogenised temperature data sets can be seen as a good representation of field scenarios.

3.4.2 Monitoring natural enemy populations

To test the validity of the degree-day based predictions describing the population dynamics of the *Neochetina* weevils and *E. catarinensis*, and indeed the temperature data on which they were based, it was essential to develop a reliable field sampling strategy that could be applied universally to all sites. When predicting *Neochetina* population size from feeding scars, Wright and Center (1984) warn that over longer periods of time, site

and seasonal effects could limit the applicability of their formula. Nevertheless, because the slopes of their regression lines between countries, among sites, between seasons, or indeed from differing sampling methods did not differ significantly, they concede that the relationship of weevil number to feeding scars was very consistent. Additionally, the authors also stress that many other biotic and abiotic factors could influence the performance of both the weevils and water hyacinth plants, therefore increasing the error inherent in applying such a model. Using the site-specific water temperature profiles from each field site provided one way to correct for such potential error. This allowed site and time specific plant growth defined by leaf production to be quantified, enabling the accumulation of feeding scars to be corrected accordingly.

Adjusting adult feeding scars in response to leaf production rates therefore preserved much of the climate induced seasonality evident in the temperature profiles at all field sites. Prior to adjustment, feeding scars were seen to accumulate on leaves during periods of slow leaf production leading to false winter peaks which indicated inflated levels of feeding and consequently, over-estimations of the number of adults present during these periods. The opposite was also true, rapid rates of leaf production allowed only limited accumulation of feeding scars on a given leaf before it was replaced by new growth, leading to an underestimation of feeding and subsequent adult population numbers during seemingly favourable periods. Adjusting feeding scars in this way corrected some of the drawbacks inherent in the 'coarse' monthly monitoring. This was evident in the fact that predicted adult population numbers correlated better with the occurrence of larval mines than with observed weevil populations. In addition to this, observed adult numbers were consistently less than predicted numbers suggesting that observed numbers were an underestimate of the real population size. This was most likely a consequence of the difficulty associated with finding the normally hidden, nocturnal adults during sampling.

Eccritotarsus catarinensis population dynamics were far less complicated owing to their fast generational turnover. Although the data were somewhat limited with mirid activity recorded from only four field sites, their population dynamics were consistent over all these sites and distinctly seasonal. Population fluctuations inferred from accumulating

feeding damage could adequately show the mirids dependency on seasonal trends. In the majority of cases, feeding damage was seen to respond closely to fluctuations in available degree-days. Nevertheless, in some cases populations appeared to undergo distinct 'boom and bust' cycles independent of the broader seasonal trends. Causal factors of these are unclear as cycles could not be successfully correlated to any particular abiotic variable. Finer scale analysis considering more acute temperature based factors such as microclimate effects and how these link directly to an organism's physiology are thus needed to better understand the variability evident in these systems.

3.4.3 Microclimate effects

Higley *et al*. (1986) warn of model inaccuracies if differences exist between the microhabitat an insect occupies and the temperature profile used to produce degree-day based predictions. Davidson *et al*. (1990) reported a 10% discrepancy between degree-day accumulations calculated from standard weather station data and those made from temperatures taken from within tomato plant canopies. In the study, weather station data collected over several years was found to both overestimate and underestimate plant canopy temperatures affecting the accuracy of degree-day models based only on weather station data. Similarly, Davis *et al*. (1996) found that site-specific models gave the best prediction of western corn rootworm (*Diabrotica virgifera virgifera* LeConte (Chrysomelidae)) adult emergence during the North American summer growing season. This effect of discrete microclimates became apparent when comparing the canopy, air, and weather station temperature profiles. These differences between discrete microclimates can be extended further and, in this study, were even recorded from within the same microclimate profile, for example due to the buffering capabilities of taller water hyacinth plants relative to short ones as evident in Fig. 3.20.

Buffering against air temperature extremes was recorded by Šajna *et al*. (2007) within the canopy of the free-floating macrophyte, *Pistia stratiotes* L. (Araceae) on a thermally uncharacteristic river in Slovenia. Due to the presence of a thermal spring, year-round water temperature in the river remained above 17°C, despite the occurrence of sub-zero air temperatures, facilitating the successful overwintering of the tropical plant outside of

its northerly distribution limits. In order to investigate the temperature gradient from water to plant and into the air layer above it, Šajna *et al*. (2007) measured water temperature, the temperature within the rosette bud, air temperature 15cm above the plant and air temperature two metres above the water surface. Using linear correlation models, temperature within the rosette bud was found to significantly correlate with water temperature, losing on average only 0.62°C when moving into the plant canopy. Temperature declined significantly further from the water/plant interface being on average 11.38°C colder 15cm above, and 17.63°C colder two metres above the plants.

Undoubtedly, the buffering of lower extremes is accentuated in the above example by the abnormally high discrepancy between water temperature and surrounding air temperature. Nevertheless, over longer periods of time, this effect could greatly influence the amount of accumulating degree-days within microclimates near the water/plant interface. Indeed, buffering of daily minima at Mkadhzi Spruit during winter 2005 allowed for an extra 11.16% and 30.40% of a single *Neochetina* and *E. catarinensis* generation respectively to accumulate over the short three month winter period relative to 2006. Another site where buffering was readily identifiable was at Kubusi River where canopy minima were on average 0.2°C per year warmer than the corresponding air temperatures. Despite being ranked as the coldest field site (Fig. 3.24) with reference to the yearly accumulation of degree-days, conditions at the site were found to be far less 'stressful' on both the insects and water hyacinth. Undoubtedly, the interplay between acute effects such as buffering, as well as broader climatic trends prevalent at the site translated into a less stressful environment. This conclusion was confirmed by the fact that *E. catarinensis* persisted at Kubusi River, and the similarly ranked Crocodile River (Fig. 3.23), throughout the study but never permanently established at either Farm Dam or Delta Park despite repeated releases.

Using the regression equation $y = -2.256 + 0.056x$ which describes the relationship between plant height and the buffering of canopy temperature minima (Fig. 3.20b), it is possible to investigate the potential effects of buffering at Delta Park by hypothetically altering petiole length. It would then be possible to show how small changes in

microclimate, for instance brought on by the effects of buffering within a deeper plant canopy, influence degree-day accumulation as well as the levels of perceived thermal stress. Simulating an average yearly plant height of 60cm at Delta Park however, produced only minor effects on both variables and didn't affect the site grouping as determined from the original principal components analysis. Negligible increases in the accumulation of degree-days (31.7 *Neochetina* and 30.1 *E. catarinensis*) per year, and only small reductions in thermal stress (*Neochetina* 9.1; *E. catarinensis* 8.2; *E. crassipes* 28.5) were evident after the simulation. Nevertheless, these small reductions in stress index values were enough to shift the Delta Park point along the factor plane of a subsequent principal components analysis of the same variables (Fig. 3.25). As a consequence of this shift the site appears more analogous to Farm Dam, a geographically

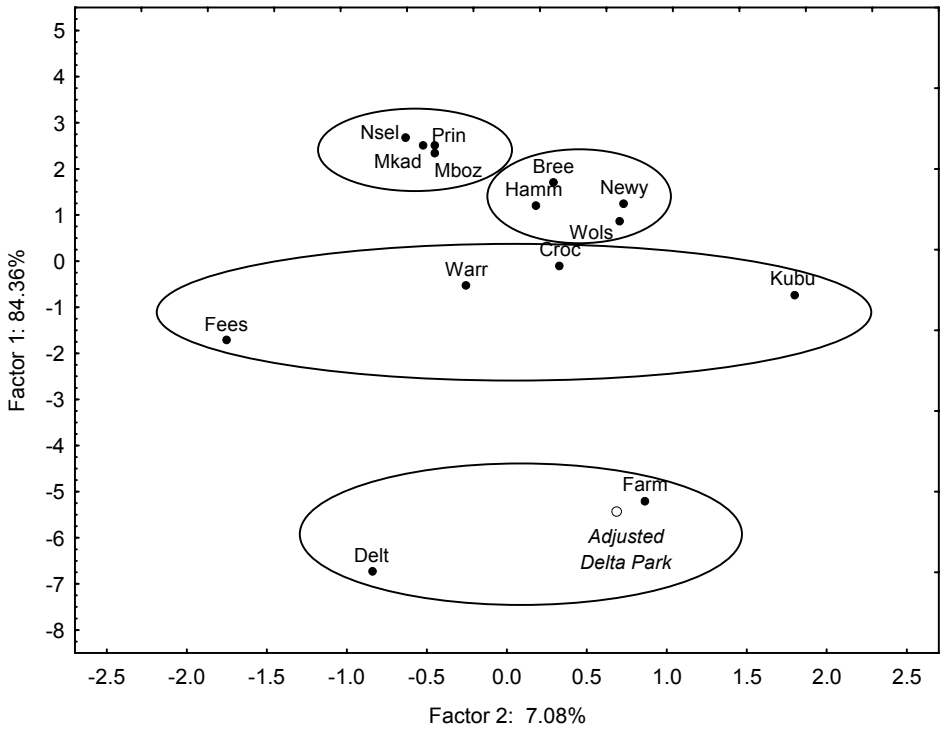


Fig. 3.25. Subsequent grouping of field sites using principle components analysis after adjustment of stress indices for Delta Park in order to incorporate the effects of simulated buffering of canopy temperature minima.

and therefore climatically similar site, but where much larger plants were found to frequently buffer lower canopy temperature extremes. It is therefore unlikely that buffering alone could make Delta Park sufficiently tolerable so as to enable, for example *E. catarinensis* to successfully establish at the site.

3.4.4 Conclusion

The above example serves to demonstrate that no single factor can be used to define an organism's existence at a site or explain its variability. Microclimate effects have a profound influence on thermal regimes, and therefore plant growth and insect activity and fecundity. Temporally, these effects vary greatly in terms of magnitude and relative importance, and are therefore difficult to model. Whilst degree-day accumulation remains a robust means of describing phenological development and generational turnover, it remains too coarse a measure to fully understand the relative importance of an organism's physiological stressors. This chapter attempts to quantify some of these effects but further work will need to be done to fully understand their implications for the biological control of water hyacinth in South Africa. Nevertheless, with the exception of a few localities, the climate over much of South Africa appears at least, to be conducive to the establishment of both the mirid and the weevils, as well as sufficiently warm so as to enable some degree of impact, but not necessarily control.

CHAPTER 4 - DISCUSSION

4.1 Out in the cold

Cruttwell McFadyen (1998) states that successful weed biological control depends on three main criteria. Namely, an agent's ability to inflict meaningful damage on a target weed, the population density achieved by a natural enemy in a novel environment, and the ecology of the target weed itself. How these criteria interact determines whether the amount of damage inflicted by a natural enemy is enough to significantly reduce the population density of a target weed, and as such, the outcome of biological control programmes. At winter limited sites monitored during this study, adverse climatic conditions were shown to hamper the biological control of water hyacinth both in terms of acute exposure to low temperature extremes, as well as prolonged exposure to low average temperatures. Insufficient temperature tolerance of the *Neochetina* weevils and *E. catarinensis* led to reduced levels of damage as a consequence of small or widely fluctuating population densities. Additionally, because water hyacinth responded differently to adverse thermal conditions, this damage in some cases was delayed, further negating the level of impact achieved.

As is readily identifiable from numerous examples in the literature, the establishment and efficacy of natural enemies introduced into cooler climates can be limited in a number of different ways. Low temperatures have been shown to prevent rapid population increase; promote high over-wintering mortality (Stewart *et al*. 1996), and natural enemy and host-plant asynchrony (Müller *et al*. 1990); slow development and feeding rates (Chown and Nicolson, 2004) and inhibit processes such as oviposition (McClay, 1996). In the majority of studies however these effects are addressed in isolation. Evaluations of the cold tolerance of the *Neochetina* weevils and *E. catarinensis* conducted during this study show that the efficacy of these insects throughout the colder regions of South Africa is hampered, to varying degrees and differentially over time, by all the above limiting factors.

The lower developmental threshold or *t* is often used as a barometer against which an organism's thermal tolerance can be gauged. Although temperatures around South Africa, especially during winter periods, frequently dropped below the estimated developmental threshold values for the *Neochetina* weevils and *E. catarinensis*, 9.6°C and 10.3°C (Coetzee *et al*. 2007b) respectively, these values could not be viewed in isolation to explain the variable efficacy of the agents, nor be used as a sole predictor of establishment in different regions. McConnachie (2004) showed that the weevil *S. rufinasus*, an extremely effective agent on *A. filiculoides* throughout South Africa, has a lower developmental threshold of 9.2°C and a developmental duration from egg to adult or thermal constant (K) of 256°D. This developmental threshold is comparable to that of the weevils and mirid and thus *S. rufinasus* can be expected to be similarly negatively affected by cold temperatures. Nevertheless, because *S. rufinasus* has a short duration of development or K, the weevil was able to complete multiple generations, on average between 10 and 12, and as many as 20 generations per year (McConnachie, 2004). This quick generational turnover undoubtedly enables the weevil to take full advantage of short favourable growing seasons in areas where winters are limiting, and build up sufficient population numbers so as to significantly damage *A. filiculoides*.

Hill and Olckers (2001) recommended that insect species with short generation times, and which are capable of rapid population increase during the short growing season of water hyacinth, should be targeted for release in South Africa. As *E. catarinensis* has a relatively short duration of development (342°D), when compared to that of the *Neochetina* weevils (984°D), the mirid's rate of population increase can be expected to be similar to that of *S. rufinasus*. Indeed, *E. catarinensis* was predicted to be able to complete multiple generations at most of the field sites monitored, based on the accumulation of degree-days. These predictions, however, did not translate into the population densities or subsequent level of impact to the plant as expected. Unfortunately, these estimations, as well as the predictions made by McConnachie (2004), do not take into account the more subtle effects of accumulating thermal stress or the acute exposure to low temperatures, examples of which range from cold-induced mortality to seasonal asynchrony brought on by differing natural enemy and target weed

ecologies. From the results obtained here, in order to begin to understand the variable population dynamics inherent between sites from differing climatic regions, the interplay between all these factors must be considered.

Bezuidenhout *et al*. (2008) stress that exposure to key behavioural and physiological thresholds or stressors, is more meaningful for determining the distribution and activity of a particular organism than simply the accumulation of heat. For example, whilst sufficient heat may be available in an environment to facilitate development, conditions may not be conducive to optimal feeding, leading to a depletion of an organism's reserves. Prolonged periods under such conditions have implications for natural enemy efficacy and eventually survival (Bezuidenhout *et al*. 2008). In addition to this, localised extinctions could also occur if aspects of an organism's reproduction, such as ovary development or oviposition, were significantly disrupted. This was particularly relevant for *E. catarinensis*. Although conducive to limited development, winter conditions at some sites were predicted to severely restrict oviposition as per the outcome of the environmental chamber experimentation. Due to the insects' short life span, the duration of these adverse conditions at some sites was expected to lead to the localised extinction of the mirid each winter. Conversely, at Kubusi River, where degree-day summation suggested that the population build-up of the mirid would be restricted to such an extent as to affect its survivability, relatively less stressful conditions enabled the insect's establishment and persistence at the site.

Quantifying the acute effects of cooler average conditions according to a set of stress indices based on observed temperatures provided an additional, possibly more realistic, or at least higher resolution means of distinguishing between field sites based on their implications for both water hyacinth and its natural enemies. Categorising field sites according to accumulating thermal stress allowed aspects of a natural enemy's efficacy to be inferred, something that degree-day summation can only coarsely hint at, and only if feeding damage is directly proportional to population growth and size. For example, nocturnal temperature trends will have a far greater affect on insects that feed and oviposit at night. Favourable daily mean temperature values would therefore overestimate

a nocturnal agent's realised level of efficacy or population growth leading to unrealistic expectations of impact. This is especially relevant for slowly developing and longer lived species where population dynamics are not necessarily governed solely by seasonal trends. As *Neochetina* population sizes are also regulated by density dependent processes and are therefore more suited to stable environments (Wilson *et al*. 2006), feeding and reproduction can therefore, potentially be drastically affected by accumulating stress independent of broader climatic trends. Additional work needs to be done to better quantify acute affects in order to determine their implications for biological control as well as to develop them as a decision making tool to direct augmentative management strategies. Furthermore, the set of stress indices used in this study is by no means complete and could be extended to encompass a variety of other relevant thresholds or restricted to better understand a single agent or life-history strategy (Bezuidenhout *et al*. 2008). The timing and number of days where water temperature precludes the onset of *Neochetina* pupation, would be of particular importance as post-winter recovery is initiated following these periods.

4.2 Modelling population dynamics

In addition to gauging the relative importance of limiting temperature factors based on their occurrence at particular groups of field sites, in order to infer an agent's survivability and potential efficacy, the use of stress indices tailored specifically to plant and insect, illustrated the differential responses to temperature of water hyacinth and its natural enemies. Through studies of an agricultural scenario, Satake *et al*. (2006) demonstrated that differing plant and insect phenologies, brought on for whatever reason, could lead to seasonal asynchrony. In this example, a lack of synchrony caused by temperature induced effects allowed tea crops to escape early season herbivory. The asynchrony between pest peak emergence and the plant's phenology was found to be a critical factor reducing the amount of crop damage. Similar asynchronous development was predicted during this study at field sites where winters were shown to be both limiting to water hyacinth growth and weevil or mirid development, but where recovery of insect and host differed. Overholt *et al*. (2009) describes a similar example where suspected asynchrony between diapause and climate negatively affected the distribution

of *Gratiana boliviana* Spaeth (Chrysomelidae), a natural enemy introduced for the control of *Solanum viarum* Dunal (Solanaceae) in the USA. *Solanum viarum* recovery after winter via root tissue and seed germination in the more northern areas of Florida occurred far later than in the south where climate was more favourable and the weed grew all year round. As *G. boliviana* diapause is largely controlled by day-length, the beetles' population build-up was anticipated to be hampered in northern Florida by food scarcity during these early season periods when weed abundance was low. Consequently, the beetle failed to establish at a number of the more northerly infestations.

Theoretical modelling of post-winter recovery of water hyacinth, the *Neochetina* weevils and *E. catarinensis* at a winter limited site was used to illustrate seasonal asynchrony. Microclimate monitoring constituting high resolution datasets enabled stage structured degree-day summation to be coupled with other temperature dependent processes such as leaf production and insect mortality. Degree-day summation was done in such a way as to integrate the physiologically relevant thermal thresholds of both the plant and insects, and could be tailored to illustrate specific life-history stages over time. The resulting model demonstrated a significant lag period between the commencement of water hyacinth post-winter recovery, and the time when the weed is subjected to meaningful levels of herbivory. Differential responses to temperature in terms of rates of population increase, and especially, relative amounts of cold-induced mortality between water hyacinth, and the *Neochetina* weevils and *E. catarinensis*, allows the weed to escape early season regulation and essentially outpace its natural enemies.

Undoubtedly, this asynchrony between insect and host will be most relevant in regions where winters are limiting. In these areas, phenological modelling will provide a means of predicting at least the first distinct post-winter early season cohorts. As winter limited sites are more likely to require supplementary control measures, these predictions isolate at least one window of opportunity to prevent the weed from outpacing its natural enemies. In this instance for example, inundative releases to boost early season natural enemy populations, or sub-lethal herbicide applications (Jadhav *et al*. 2008; Byrne *et al*.2010) would severely hamper the post-winter recovery of water hyacinth. However,

isolating these sorts of events from the long-term field site profiles proved to be difficult. This was due to high levels of variability between, as well as within sites over time, the unpredictable occurrence of stochastic events such as flooding and frost, and the interplay between various acute factors influencing microclimate, for example petiole length. Subsequent cohorts or generations become less prominent and therefore more difficult to predict further from winter.

In broad terms, the activity of the *Neochetina* weevils and *E. catarinensis* was seasonal at all field sites, with both feeding and reproduction highest during the more favourable summer months. Although this trend was prevalent at warmer sites, namely Mkadhzi Spruit, Mbozambo Swamp and Nseleni River, distinct generation cohorts or peaks could not be readily distinguished for any agent at any time. This was undoubtedly brought on by year-round favourable conditions resulting in multiple overlapping generations per year. This was not the case at interior, high-altitude sites where winters and frost negatively affected both water hyacinth growth and insect activity and survival. Wilson *et al*. (2001) speculate that winter or other such severe events have the potential to synchronise the life-history stages of an insect population if the event affects survivability. Supporting this is the fact that distinct generational cohorts, at least in the case of the slower developing weevils, become more apparent at colder sites. *Neochetina* populations therefore appear to be synchronised each year by winter. This is inevitably brought on by the high mortality among early instar larvae predicted as a consequence of frost, as well as a lack of oviposition coupled with the adult mortality prevalent in winter. *Neochetina* populations emerging from winter are therefore synchronised and dominated by third instar larvae, as outlined in the post-winter recovery model.

Although the synchronisation of life-history stages increases the predictability of certain phenological events, and is thus more conducive to timed management intervention, it also has the potential to make insect populations more susceptible to disturbance. The magnitude of this susceptibility will vary depending on whether the generation of a natural enemy is long relative to the time scale and frequency of disturbances (Wilson *et al*. 2001). Indeed, disturbances were seen to have a profound influence on the natural

enemy populations at a number of sites throughout field sampling. At the colder field sites, *Neochetina* generation turnover was slow and generations were seen to straddle seasons. An early season *Neochetina* population dominated by early instar larvae present in the aerial parts of plants would be especially vulnerable for example to the effects of frost or herbicide applications resulting in leaf die-back. Stochastic events, in particular, related to frost and winter, have the potential to reset weevil populations to low starting sizes at least annually, and in some cases more frequently. Whilst water hyacinth was generally quick to recover following such disturbance, the weevils were far slower, in some cases taking an entire growing season to return to population levels comparable to that prior to disturbance. A good example of this occurred at Crocodile River where a flood removed the majority of plants at the site in 2006. Plants were quick to resurge but remained largely free from the effects of the weevils for the next seven months. In this way, only stable sites free from disturbances, including adverse winter conditions, will be able to sustain consistently high weevil populations and thus, be most conducive to effective biological control. Unfortunately this was evident at only one site, namely Nseleni River, but also to a certain extent at Mkadhzi Spruit.

4.3 Integrated management

With the exception of a few localities, the results of this study show that the climate over much of South Africa is conducive to the establishment of both the mirid and the weevils, as well as sufficiently warm so as to enable some degree of impact, at least during the more favourable summer periods. Nevertheless, due to the numerous limiting factors brought on by adverse climatic conditions and a lack of stability, biological control alone is unlikely to effectively manage water hyacinth at a large proportion of field sites and therefore integrated management needs to be emphasized. Any management strategy however, will have to be closely synchronized with the site-specific seasonality inherent in both water hyacinth and its natural enemy phenologies. For example, Wilson *et al*. (2005) stress that because density dependence in early instar larvae is a crucial factor in regulating *Neochetina* populations, they will be very sensitive to changes in leaf dynamics. Control options such as herbicide application could thus have the potential to seriously undermine the control facilitated by the weevils if mistimed. Indeed, Center *et*

al. (1999) found that *Neochetina* populations at water hyacinth infestations subjected to long-term, largely ad-hoc maintenance management via chemical applications, were significantly reduced relative to populations at unmanaged sites. The reduced natural enemy developmental, and feeding rates in response to temperature demonstrated throughout this study also indicate that any realised impact of biological control in cooler regions is likely to take considerably longer to achieve compared with warmer regions. Hill and Olckers (2001) speculated that successful biological control in cooler areas of South Africa could take considerably longer, between 8-10 years, than in regions with more favourable climatic conditions where control is normally achieved in around four years (Julien *et al*. 1999). Although this may be the case in less extreme regions of the country, climate constraints are anticipated to completely preclude successful biological control at some sites. Augmentative management, including repeated releases of biological control agents at such sites will be required *ad infinitum* (Julien *et al*. 1999). Unfortunately, delays associated with this sort of approach are often perceived by water authorities to be unacceptably long when compared with pure herbicidal or mechanical controls (Julien *et al*. 1996, Hill and Olckers, 2001). Inevitably then, managers confronted with the urgency of control, turn to 'quick-fix' solutions which often over-ride decisions conducive to long-term control and have the potential to disrupt natural enemy populations (Julien *et al*. 1996). Phenological models, in these situations, will be useful for managing stakeholder expectations of biological control impacts across sites with differing climates.

This study has shown that key stages in terms of vulnerability of both water hyacinth and its natural enemies can be accurately predicted via their temperature dependent processes. However, fine-scale temperature measurements from different microclimates were essential to underpin these models. Herms (2004) concedes that whilst researchers are able to develop highly accurate models by recording temperature directly in an insect's environment, these models are restricted in terms of their practicality as a viable management tool. In essence, temperature profiles used to develop such models will differ enough from profiles normally available to management practitioners, such as temperature records obtained from nearby weather stations, that the value of such models

will be limited or have to be applied with caution. Additional work will thus need to be undertaken in order to make these types of models more applicable to broader field situations if key phenological stages are to be accurately predicted. Work done here already showed good agreement between fine-scale microsite temperature data and offset weather station data, suggesting that this will therefore be possible. Nevertheless, potential offsets will need to be carefully tailored to specific sites based on conditions prevalent at the time, for example plant size in order to adjust for processes such as buffering of minimum temperatures.

4.4 Conclusion

This study represents a critical analysis of one abiotic variable affecting the biological control of water hyacinth in South Africa. Whilst temperature induced effects are numerous and have widespread implications for both water hyacinth growth and the realized impact of its natural enemies, these effects will also be accentuated or mitigated by the influence of other biotic and abiotic variables. A more holistic view, for example, incorporating the interspecific effects of the other agents, as well as water nutrient regimes and hydrological features such as flow and depth, must therefore be taken in order to fully appreciate the variability and complexity inherent within water hyacinth systems around South Africa.

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