SPATIAL AND TEMPORAL OCCURRENCE OF FORENSICALLY IMPORTANT SOUTH AFRICAN BLOWFLIES (DIPTERA: CALLIPHORIDAE)

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ABSTRACT

Forensic entomology is an emerging field in South Africa. Little is known about South African blowflies and factors that affect their use in a forensic context. This work provides a review and synthesis of previous work in South Africa and supplements some of the background and basic knowledge required for forensic entomology in South Africa.

The seasonal occurrence of eight forensically important blowfly species was quantified by fortnightly trapping in Grahamstown, South Africa. The spatial distribution of each species was related to seasonal occurrence and habitat preference. Seasonal distributions of blowflies in carcasses in South Africa were obtained from the literature and compared to the seasonal trapping.

By mapping South African locality records of forensically important blowflies and analyzing these records in a modified Principal Components Analysis of climatic data, the potential geographic distributions of each fly species was modelled. Most species were widespread, but *Calliphora croceipalpis*, Jaennicke, 1867, was found in cold places. This information is important for determining where certain species are likely to occur in forensic investigations.

Nocturnal oviposition was examined in both field and laboratory experiments. *Lucilia* species could oviposit nocturnally in the field, while *Lucilia* species, *Chrysomya chloropyga*, (Weidemann, 1818) and *C. putoria* (Weidemann, 1830) could oviposit nocturnally in the laboratory. These findings are important factors in affecting the precision of estimates of a post mortem interval (PMI) by up to 12 hours.

The thermophysiological ranges of four species of adult blowflies were determined by measuring onset temperatures of four significant behaviours: onset of neural activity; onset of coordinated movement; shade-seeking and death. There was a sexual size dimorphism in *Lucilia* species, *Chrysomya chloropyga* and *Calliphora croceipalpis* with females being larger than males. *Chrysomya megacephala* (Fabricius, 1794) had an unexpectedly high death threshold, while *Calliphora croceipalpis* had the lowest death threshold of the flies tested. These points were related to the seasonal and geographic occurrence of each species, to nocturnal activity and placed in a forensic context.

To Allan, Linda, Liesl and Joanne

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PREFACE

This work was born out of an interest I developed in Forensic Entomology in my third year at university. The lack of information available on South African insects for use in Forensic Entomology was a driving force behind this work.

I would like to thank, most sincerely, my supervisor and mentor, Professor Martin Villet, for all his assistance, ideas, encouragement and support. Few people are as dedicated to their students and their work.

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CHAPTER 1

Introduction

The history of forensic entomology research in the northern hemisphere has been documented in a variety of recent reviews (Erzinclioğlu, 1983; Keh, 1985; Smith, 1986; Catts, 1992; Catts & Goff, 1992; Amendt, 2000; Benecke, 2001; Snyder Sachs, 2002). Forensic entomology has rarely been the focus of work elsewhere, but because of the significance to man of necrophages, there is a wealth of published research that is relevant to the topic. The aim of this introduction is to provide an historical review of the situation in South Africa and place this work in context.

In 1921, R.W. Thornton, Principal of the Grootfontein School of Agriculture, convened a committee to investigate sheep strike in South Africa. At this stage wool was the most important export after gold in South Africa. The work commenced in September 1922 when Owen Wahl, an entomologist from Grootfontein, returned from Australia and the United States where he had gathered information on dealing with sheep strike. Many of the foreign measures were not feasible in South Africa and it was realised that a great deal needed to be learned about South African blowflies and their habitats. In 1923 Wahl was transferred and Bernard Smit continued this work.

Smit (1927, 1931) specifically investigated the seasonal distribution, life-history and parasites of the South African blowflies. His main objective was to find methods of controlling blowflies to prevent sheep-strike. Smit did not recognise *Lucilia cuprina* (Weidemann, 1830) and suggested that *Chrysomya putoria* (Weidemann, 1830) was only found further north in Africa. This lack of clarity in the systematics makes some of his work difficult to interpret. Smit concluded that crutching sheep was the best way to protect them from attack and that burying carcasses was not an effective measure in reducing sheep blowflies.

A decade later G.A. Hepburn (1943a, b) investigated the role that carcasses play in nature as sources of blowflies. This work was a collaboration done at Onderstepoort under H.O. Mönnig, who was the head of Parasitology. This work was also pursued in response to myiasis in sheep that continued to be a big problem. A survey was conducted to discover which flies farmers were experiencing trouble with across the

country. Hepburn's main objective was to find what species of flies bred in carcasses under field conditions, as Smit had already done.

Mönnig & Cilliers (1944) carried out similar work to Hepburn, but at Bredarsdorp in the winter rainfall area. Results similar to Hepburn's (1943a, b) were obtained in this study but an attempt to correlate rainfall and blowfly abundance was not successful. Suggestions were made to bury carcasses in an attempt to control blowfly populations, which was contrary to what Smit (1931) had stated.

George C. Ullyett's (1945) work on *Lucilia* genetics cast doubt on the identity of the species in South Africa. It suggested that *L. sericata* (Meigen, 1826) and *L. cuprina* readily interbred and that these hybrids were fertile.

Working for the Department of Agriculture, Ullyett (1950) was interested in how competition affected the blowfly populations in carcasses and how exactly relationships between species were responsible for the reduction in fly populations under given conditions. He hoped that this might provide biological control. He studied development rates of blowflies, in particular *L. sericata* and *Chrysomya chloropyga* (Weidemann, 1818). He concluded that both intra- and interspecific competition occurred on carrion and that *Lucilia* was the most capable of surviving adverse conditions. He also concluded that destruction of carcasses was not sufficient to reduce blowfly populations and that a great deal more needed to be known about the relationships between populations, their environment and natural controlling factors to control blowflies, specifically in the light of sheep strike. His work was impressive, but unfortunately it ceased when he left for Canada in 1949.

Fritz Zumpt and P.M. Patterson (1952) reported on the seasonal abundance of flies attracted to dead guinea pigs, beef and human faeces. The concern of this study was the spread of diseases, particularly poliomyelitis, by flies. This work was conducted in Johannesburg for the South African Institute for Medical Research for the Department of Mining, in relation to the health of migrant labourers. Zumpt also reviewed the systematics of blow- and fleshflies (Zumpt, 1956, 1972) and myiasis in man and animals in the Old World (Zumpt, 1965). These works contain illustrated indentification keys. He did not recognise *Chrysomya putoria* as a separate species.

There was a lull that was broken by a series of academic studies.

Hugh E. Paterson's (1968) work for his doctoral thesis at the University of the Witwatersrand concerned the evolutionary and population genetics of certain Diptera. Of interest was his chapter on *C. putoria* and its evolutionary status. He conducted various experiments to determine whether *C. chloropyga* and *C. putoria* were separate species, and concluded that they are distinct biological species.

Dr Fredrik W. Gess, assistant entomologist at the South African Museum in Cape Town from 1959 – 1968, was on several occasions asked to assist the state pathologist with identification of maggots found on corpses. He was trained in this work by Dr. A.J. Hesse, a dipterist, who had previously undertaken forensic investigations. The aim of this work was to corroborate the pathologist's estimate of time since death. Gess was never required to testify in court and provided his evidence in written reports. In 1968 he joined the Albany Museum in Grahamstown and André Prins took over from him (pers. comm.).

Prins (1979), working at the South African Museum in Cape Town, reported the first records of *C. megacephala* (Fabricius, 1794) in South Africa. This species was identified in 1978 breeding in seagull carcasses at Ysterfontein in the Western Cape. As this species is forensically important, the finding was very valuable.

Paterson's student, Ivan Meskin (1980), specifically studied the morphology, biology and ecological studies of the highveld blowflies and the factors affecting their co-existence for his MSc. The niche of each species was studied, delineated and related to the co-existence of these species in a community. He found species to be separated by habitat preference, seasonal succession, ecological succession on carcasses and different larval feeding habits. He also conducted extensive work on egg and larval identification of the blowfly species of the highveld. The specialisation of each species to allow co-existence was placed in an evolutionary context. His work on the co-existence of blowflies on the highveld was published in 1986 and his work on egg morphology in 1991.

Laurence E.O. Braack (1981, 1986, 1987) studied the decomposition of carcasses and the associated fauna to elucidate the role that decomposers play in ecosystems. A large part of this work was conducted in the Kruger National Park. Braack (1984) described epidermal streaming, a term to describe the process whereby maggots get under the skin of a dead animal. Braack & de Vos (1987) also looked at the seasonal abundance of blowflies in the Kruger National Park to assess their significance in the spread of anthrax. In 1991, Braack acknowledged that he had misidentified *C. megacephala* as *C. bezziana* Villeneuve 1914, in the Kruger National Park in his study in 1984. This led to speculation about how *C. megacephala* had spread so rapidly across South Africa. Braack & Retief (1986) estimated dispersal rates of *C. megacephala* and *C. marginalis* (Weidemann, 1830) to be 2 – 3km/day.

Prins (1982, 1983, 1984a, b) published a series of papers on decomposition and the associated arthropods based on thesis work. Of particular forensic interest was his paper on the morphological and biological aspects of six South African blowflies (1982). His studies were specifically aimed at the forensic context as no work of this kind had been done in South Africa and had been requested by the police and State Health Department regarding cases of murder and cattle theft (Prins, 1983). This was therefore the first study in South Africa that was explicitly for use in a forensic context.

In the past decade, Theunis van der Linde, a medical entomologist at the University of the Free State formed a group called FEITOVS that studied deaths that provided work for forensic entomologists (Louw & van der Linde, 1993). In a series of conference abstracts, Van der Linde and his students have reported on work on succession with particular reference to burned and frozen carcasses (van der Linde & Hugo, 1997; Robberts & van der Linde, 2001), shaded versus sunny carcasses (van der Linde & Leipoldt, 1999), hanging carcasses (Kolver & van der Linde, 1999; Kolver *et al.*, 2001) and covered carcasses (van Wyk *et al.*, 1993; Cronje *et al.*, 1995) They have also looked at using maggots as toxicological material (van Wyk & van der Linde, 1995) and at development rates of maggots (Leipoldt & van der Linde, 1993; Stadler & van der Linde, 1995). Martin H. Villet and his research group at Rhodes University have published on forensic toxicology (Musvasva *et al.*, 2001) and have designed an electronic key, IdentiFly, to help in fly identification (Bownes *et al.*, 2000). Work has

also been conducted on sequencing DNA of certain species of blowfly as well as work involving the development rates of maggots.

Forensic entomology has started to come into its own in South Africa. Evidence of this is that Mervyn Mansell of the Plant Protection Research Institute of the Agricultural Research Council has been helping the police with cases as a forensic entomologist and has testified in court (Gunnel, 2000; Groenewald, 2002).

Although much of the work done on blowflies in the past was not in a forensic context, certain aspects of the work can be used: Smit's (1931), Hepburn's (1943a), Mönnig & Cilliers' (1944) and Zumpt & Patterson's (1952) seasonal distribution work; Ullyett's (1950) seasonal distribution work and development rates of maggots; Zumpt's (1956, 1972) identifications and keys; Prins' (1982) morphology of six South African blowflies and Braack's (1981, 1986, 1987) succession work.

The lack of overt research in a forensic context on blowflies in South Africa means that there is vast scope for study. Work has been geographically localised in the Western Cape and Gauteng. Work in the Eastern Cape is too old to be taxonomically reliable and no work has been done in KwaZulu Natal or the Northern Cape. The aims of this study were therefore to revise, refine and extend some of the fundamental data required for forensic entomologists to function efficiently in South Africa. These include seasonal distribution, nocturnal oviposition, adult temperature tolerance ranges and geographic distribution. Although neglected by past research, these issues are pivotal to estimating post mortem intervals (Keh, 1985; Smith, 1986; Greenberg, 1990; Catts, 1992; Catts & Goff, 1992).

CHAPTER 2

Seasonal and spatial distribution of forensically important flies in Grahamstown, Eastern Cape, South Africa

INTRODUCTION

The prime responsibility of a forensic entomologist at a death scene is to determine a post mortem interval (PMI) using the insect fauna present on a body (Hall, 1990; Catts,1992; Wells & LaMotte, 2000; Benecke, 2001). The use of maggots by forensic entomologists as indicators of time of death is influenced by many factors such as temperature, climatic zones, season and the presence of toxins (Erzinclioğlu, 1983; Catts, 1992; Wells & LaMotte, 2000). Seasonality is of particular interest in South Africa as the information available was collected for other purposes than for use in a forensic context, and the records are dated (Smit & du Plessis, 1926; Smit, 1931; Hepburn, 1943a; Mönnig & Cilliers, 1944; Ullyett, 1950; Zumpt & Patterson, 1952; Braack & de Vos, 1987). The introduction and spread of *Chrysomya megacephala* for example has occurred in the last 20 years in South Africa and its occurrence is therefore not reported in older studies. It is very important to have an accurate record of the seasonal distribution of forensically important flies in South Africa if they are to be used by forensic entomologists. This information will also be useful for veterinary and public health issues.

In previous studies on the seasonal distribution of blowflies in South Africa (Smit & du Plessis, 1926; Smit, 1931; Hepburn, 1943a; Mönnig & Cilliers, 1944; Ullyett, 1950; Zumpt & Patterson, 1952; Braack & de Vos, 1987) the focus was on flies that cause sheep-strike or spread diseases. Very little attention has been paid to the forensic importance of the flies in South Africa. Numerous decomposition studies have been conducted in other countries (Fuller, 1943; Chapman, 1955; Bornemissza, 1957; Walker, 1957; Reed, 1958; Payne, 1965; Cornaby, 1974; Johnson, 1975; Lane, 1975; Coe, 1978; O'Flynn & Moorhouse, 1979; Greenberg, 1990; Shean *et al.*, 1993; Tantawi *et al*; 1996; Davies, 1999; Cento *et al.*, 2002). Particular attention has been paid to the ecological succession of insects associated with decay, with seasonality being studied in more detail in recent years (Johnson, 1975; Denno & Cothran, 1975; de Souza & Linhares, 1997; Davies, 1999; Lopes de Carvalho & Linhares, 2001).

Ecological succession in a carcass varies with seasonal changes in the blowfly community (Fuller, 1934) and thus, when examining succession, it is important to know what blowflies are present at what time of year.

MATERIALS AND METHODS

Eight sites within and around Grahamstown were chosen to represent urban and semi-urban areas (Fig. 2.1). The urban areas were further divided into developed areas in residential gardens and semi-developed areas in Grahamstown East (township). The semi-urban sites were in the small industrial area where human activity occurs, but is less prominent. These sites were chosen to cover the range of habitats that flies could experience within the Grahamstown municipal area.

Site 1 was at the riding club where numerous horses are stabled with open ground surrounding them. This site is within 200m of the municipal rubbish dump (Fig. 2.1). Site 2, the municipal Traffic Department offices in the industrial area, is surrounded by small industrial facilities and farming land. Site 3 was at the Tick Research Unit facilities of Rhodes University. This site is beyond the university residence areas and is surrounded by open land. A few people live here and dogs, goats, chickens and pigs are kept. Sites 4 - 6 were in the middle-income residential area of Grahamstown. They had, respectively, two dogs; 4 dogs and a cat, and a dog and geese. Sites 7 and 8 were in the Grahamstown East (Rhini) township in a poor, high-density residential area. Dogs and cats were common and domesticated animals such as donkeys, cattle, goats and chickens were common in both areas. There was also refuse strewn in the streets and open plots.

Eight Redtop fly traps (Miller Methods, Ltd.) were modified by removing the base of the traps and attaching screw-top bottles to them. The centres of the lids were cut out and the opening of the bottles covered in netting. The bottles were then screwed onto the plastic base of the traps with the lids. This allowed flies to detect the odour of the bait and enter the trap, but prevented them from getting into the bait and thus being fouled by it. The flies were therefore trapped in the bag of the trap and were easily removed. At each trapping site, a metal pole 1.2m long with a horizontal arm of 0.2m

at the top of the pole was planted into the ground to standardise the height at which the traps were placed.

Each trap was placed so as to receive maximum sunlight during the day. This varied slightly between the sites due to orientation of houses and trees, and between seasons.

Traps were placed in the field for 4 days at fortnightly intervals from the beginning of May 2001 until the end of June 2002. Approximately 125g of chicken liver was placed in each bottle for every trapping session. At the end of the 4-day period, the traps were brought back to the laboratory, where the flies were chilled in a walk-in fridge at 4°C before being removed and placed in 95% ethanol. The flies were then identified using Zumpt (1956) and Holloway (1991) and the numbers of the different species were recorded. When samples became too big for efficient sorting of the whole sample, they were split using a modified Folsom splitter (Postel *et al.*, 2000) This was similar to a technique used by Murray (1956) that yielded satisfactory results. Each whole sample was checked for rarer species.

Each species was analysed using 2-way ANOVA to determine significant differences in monthly abundance and between traps. Post hoc multiple range tests were used to identify which months and traps were statistically different. Rainfall and temperature data for the trapping period for Grahamstown were obtained from the South African Weather Service (Fig. 2.2).

RESULTS

The climate was warm in summer, cool in winter and had bimodal rainfall for the trapping period (Fig 2.2). The rainfall in June 2002 was unusually high.

Clear seasonality exists in the blowfly populations in the Grahamstown region (Figs. 2.3 – 2.11).

Lucilia sericata (Fig. 2.3) shows a clear summer abundance, reaching maximum numbers between November and January. This peak was statistically significant (Table 2.1). However, it was present throughout the year with minimum numbers

occurring between July and September. *L. sericata* was caught most often at the two sites in Grahamstown East and the site at the stables. There was a significant difference in samples between site 7 and the other trapping sites. No interaction between trapping site and month was noted (Table 2.1).

Lucilia cuprina (Fig. 2.4) had a summer peak that was statistically significant (Table 2.1), reaching maximum numbers in November and December. It was present throughout the year in smaller numbers, with the lowest numbers in July and August. There were significant differences between sites 1 (stables), 7 and 8 (Grahamstown East) and the other trapping sites. No interaction between the trapping site and month was noted (Table 2.1).

Chrysomya marginalis (Fig. 2.5) had a summer peak, obtaining maximum numbers in November and a second, smaller peak in March. There was a significant difference between the months, but no significant difference was found between the traps (Table 2.1). Low numbers of *C. marginalis* were recorded between July and September. No interaction between the month and the traps was recorded (Table 2.1).

Chrysomya albiceps (Weidemann, 1819) (Fig. 2.6) was caught in large numbers from October to January and again in March. The summer peak was significantly different to the other months (Table 2.1). There was also a peak in June 2001. No significant difference between the traps or interaction between the month and traps was noted (Table 2.1).

Chrysomya chloropyga (Fig. 2.7) reached maximum numbers in November. This species was trapped in large numbers from August to December with virtually no flies being caught from February to May. Site 1 (stables) was significantly different to the other trapping sites in November (Table 2.1). The interaction between month and trap was significant because of this sample (Table 2.1).

Chrysomya putoria (Fig. 2.8) peaked later than *C. chloropyga*, reaching maximum numbers between December and January and peaking again in slightly lower numbers in March. The summer peak was significant (Table 2.1), while there was a significant difference between sites 1, 2 and 3 and site 7, and between sites 1, 2, 3, and 4 and site

8. There was also a peak in June 2001. No interaction between month and site was recorded (Table 2.1).

Chrysomya megacephala (Fig. 2.9) showed a distinct preference for cooler weather, peaking in May and June. The winter peak was significant (Table 2.1), while low numbers of this species were caught from November to March. There was a significant difference between sites 2, 3, 4, 6, 7 and 8 and site 1, but no interaction between month and site was noted (Table 2.1).

Calliphora croceipalpis Jaennicke, 1867 (Fig. 2.10) was the only species of fly that did not show significant seasonality (Table 2.1). This species was trapped in low numbers from May 2001 to December and again from March to June 2002 with a small peak between September and October. No significant difference between trapping sites or interaction between site and month was recorded (Table 2.1).

Sarcophagids (Fig. 2.11) were trapped in lower numbers than the blowflies, but were consistently present. There was a definite summer peak from October to April that was significant (Table 2.1). Lower numbers were trapped in the cooler months. A significant difference was found between sites 5 and 6 and site 8, but no interaction between site and month was noted (Table 2.1).

DISCUSSION

Blowfly census techniques

As Ullyett (1950) pointed out, decomposing material is only attractive to particular species of flies for certain periods of time. This means that any trapping method that does not take this into account may be lacking certain species, not due to their absence in the field, but rather to the lack of attraction to the bait. In this study the traps were baited with fresh liver and left for 5 days in the field. This method appears to be effective because all the forensically important flies were trapped at some point during the 14 months of trapping. This suggests that the bait decomposed sufficiently in 5 days to be attractive to all the relevant species.

Braack and De Vos (1987) suggested that the quantity and type of bait are essential for effective trapping of species. Baits of at least 500g were suggested to be necessary

to ensure trapping of species such as *Chrysomya marginalis*. This study found *C. marginalis* to be attracted to smaller baits, although in lower numbers. Ullyett (1950) suggested that baits needed to be exposed regularly during a month to prevent artefacts due to changes in the weather and natural fluctuations in the fly populations from influencing results negatively. Fuller (1934) expressed similar concerns, but stated that when the numbers of flies caught per month were presented as graphs, these variations due to weather and activity of the flies are smoothed out, thereby giving a representative picture of the seasonal abundance of the different species. In this study, there was a disparity in the numbers of flies from June 2001 to June 2002, which can be attributed to a particularly warm week in which trapping took place in 2001 and heavy rain in 2002 (Fig. 2.2). However, the seasonal trend is still evident, particularly for species like *Chrysomya albiceps* (Fig. 2.6). Thus, placing traps for 4 days every alternate week as in this study, should give a reliable indication of the relative seasonal occurrences of all the forensically important species despite year-to-year seasonal fluctuations.

Although lower numbers of blowflies could be expected during winter, the lack of certain species may not be due entirely to their absence at this time of year, but rather because the bait did not decompose sufficiently in the 4 day period to be attractive to certain species. However, the trends observed in this study concur with previous studies (Smit, 1931; Zumpt & Patterson, 1952; Linhares, 1981; Braack & De Vos, 1987; De Souza & Linhares, 1997), suggesting that five days is longer than the "window of attraction" (Catts, 1992) for South African species, even in winter.

The results of the work by de Vries (cited in Ullyett, 1950) on carcasses do not agree with the seasonal results obtained in this study. De Vries & Hepburn's (1943b) work suggests that *Lucilia* spp. are absent during summer and breed abundantly in carcasses during winter. This study and several others indicate that *Lucilia* spp. were present in large numbers only in summer (October – March). Similarly, *C. chloropyga* is recorded by de Vries as being present in carcasses in summer in only very small numbers, but in this study and others (Table 2.2) it is present in very large numbers from September to December. This suggests that obtaining seasonal abundances from carcasses and from trapping gives very different trends. The reason for this, Ullyett (1950) suggested, is that the larval populations on carcases give rise to the adult fly

populations, which thus peak later. This is seen by comparing the trapping results of Smit (1931) with the carcass results of de Vries (cited in Ullyett, 1950). The seasonal trends that Smit (1931) recorded (Table 2.2) are similar to those of this study. *Lucilia* spp. and *C. chloropyga* have a summer peak despite the higher numbers in carcasses recorded in winter by de Vries. Although Ullyett's explanation holds for these species, *C. albiceps* and *C. marginalis* do not follow this trend. For these two species, the carcass and trap populations coincide very closely (Ullyett, 1950). Smit (1931) claimed that *C. albiceps* does not lay eggs in winter and Tantawi (1996) suggest that *L. sericata* breeds in winter to prevent competition with *C. albiceps* larvae that are predatory. Caution must be therefore taken with census methods when trying to ascertain seasonality of adult flies. Ullyett (1950) suggested that the density of the parent population when breeding in carcasses occurs, must be roughly proportional to the density of the population of the filial generation it produces. This is not necessarily the case as one female *Lucilia* can lay 180 - 380 eggs (Ullyett, 1950). Thus a few flies can give rise to thousands. This means that a large number of adult flies may arise from a carcass from just a few parental females and thus bias trapping results.

Because the apparent timing of peaks in fly abundance differs depending on whether they are carcass- or trap-based, only studies that used traps will be compared in this study.

Another factor that affects population estimates when using carcasses is the carcass size. Different size carcasses attract different flies (Denno & Cothran, 1975). Thus, seasonal work carried out on carcasses that differ in size, may not provide an accurate representation of the flies in the field at that time. This is another reason for leaving out seasonal carcass work such as Hepburn's (1943b) and Meskin's (1986).

Mönnig & Cilliers (1944) suggested that since *Lucilia cuprina* strikes sheep, meat baits would not be very efficient at attracting this species. Thus the proportion of the *L. cuprina* trapped is not representative of the flies present in the area. It is debatable how efficient different baits might be at attracting specific species but this does not necessarily imply that all studies are therefore useless. This study is not concerned with the proportion of the different flies in the community, but rather the absence or

presence of particular species at certain times of the year. Thus, although the number of some species may be low (e.g. *C. marginalis, L. cuprina*), as long as the census method is used consistently, their seasonal trend is obvious and in most cases comparable to previous studies.

Seasonal trends in the populations

The rainfall (Fig. 2.2) recorded in the Grahamstown municipal area does not show a distinct seasonality although there is a bimodal trend (Fig. 2.2). No interaction between rainfall and temperature is apparent and as such the rainfall does not appear to affect the occurrence of blowflies. However, although no interaction is obvious, in general when it rains in Grahamstown the temperatures drop below the average for that time of month. This change in temperature may have a transient effect on the numbers of active blowflies.

Several people have examined seasonal distribution of blowflies in South Africa relative to the spread of disease and sheep-strike (Table 2.2): Smit (1931) at Grootfontein in the arid Karoo; Hepburn (1943a) at Onderstepoort and Zumpt & Patterson (1952) in Johannesburg in temperate savannah; Mönnig & Cilliers (1944) at Bredarsdorp in the Mediterranean climatic region of the Western Cape; and Braack & De Vos (1987) in the Kruger National Park in subtropical bushveld. There have also been many similar studies carried out in other countries (Table 2.3). Some of these are of comparative value as they include some of the species found in South Africa – Fuller (1934) in Australia; Williams (1953) in New York, USA; Schoof & Savage (1955) in 5 different towns in USA; Murray (1956) in New Zealand; Linhares (1981) in Campinas City, Brazil; and Martinez-Sanchez *et al.* (2000) in Spain.

Lucilia sericata (Fig. 2.3) is a cosmopolitan species that has been recorded in all but one of the abovementioned studies (Tables 2.2 & 2.3). From these results, *L. sericata* is found to peak during the summer months with minor yearly fluctuations. It appears to be limited by the minimum temperatures in winter (Fig. 2.3).

Lucilia cuprina (Fig. 2.4) has often been grouped with *L. sericata* (Smit, 1931; Mönnig & Cilliers; 1944, Braack & De Vos, 1987) due to lack of taxonomic separation either through ignorance or lack of knowledge. This species therefore is

considered to have much the same seasonal distribution as *L. sericata* (Smit, 1931; Mönnig & Cilliers; 1944; Braack & De Vos, 1987). Results of the preset study show that *L. sericata* and *L. cuprina* have very similar seasonal distributions (Figs. 2.3 & 2.4). Two studies refer specifically to *L. cuprina* as active from July – September (Hepburn, 1943a) and peaking in August and again in December with numbers decreasing towards March (Linhares, 1981). This species seems to be limited by the minimum temperature in winter and the maximum temperature in February to April (Fig. 2.4).

Chrysomya marginalis (Fig. 2.5) appears to have a distinct summer abundance (Table 2.2). This species was caught in low numbers that may be the result of a preference observed by Hepburn (1943a) for carcasses over traps. It also appears to be limited by the minimum temperature in winter (Fig. 2.5).

Chrysomya albiceps (Fig. 2.6) was present most of the year, but not in great numbers from July – September. The results obtained are similar to those from other parts of South Africa (Table 2.2). There is a notable dip in February that may be attributed to excessively high temperatures and low rainfall during these trapping weeks. Extremely high temperatures during a previous study were believed to be responsible for a decrease in fly numbers in February (Smit & du Plessis, 1926). Flies in an insectary died when exposed to extreme heat and this appears to explain the general fall in fly numbers in February that then increased again in March – April when the temperature is cooler.

Chrysomya chloropyga (Fig. 2.7) was trapped in large numbers, which Smit (1931) also experienced. The summer peak in November is comparable to the results obtained by other authors (Table 2.2). However, Linhares (1981) found *C. chloropyga* occurring in Brazil in January – April as well as in August – October (Table 2.3). Flies were not recorded from February to May during the present study. This may be due to seasonal or latitudinal differences between Brazil and South Africa. This species also appears to be limited by maximum and minimum temperature extremes (Fig. 2.7).

Chrysomya putoria (Fig. 2.8) has a seasonal distribution that appears to be limited by the minimum temperatures in winter. Braack & De Vos (1987) found similar seasonal trends to this study (Table 2.2).

Chrysomya megacephala (Fig. 2.9) shows a distinct winter distribution. Linhares (1981) recorded this species in August and low numbers from April – July.

Calliphora croceipalpis (Fig. 2.10) did not exhibit a significant seasonal trend. The slight peak in October is similar to other studies (Table 2.2). This species was not trapped in January – March suggesting that it occurs year round with the exception of the hottest months when the maximum temperatures are above 25°C (Fig. 2.10).

Sarcophagids (Fig. 2.11) show a trend for being present in larger numbers in summer and lower numbers in winter. Although not caught in great numbers, they have the potential for being important, particularly when small carcasses are present (Denno & Cothran, 1975).

Spatial distribution

Lucilia sericata and *L. cuprina* were trapped most often at the stables (site 1) and the two sites in Grahamstown East (sites 7 & 8). At all three of these sites there are livestock, ranging from horses, cows and donkeys to goats and chickens. This may explain the greater relative abundance as there is abundant manure that may act as protein sources or even breeding material (Lopes de Carvalho & Linhares, 2001). The lack of a significant difference between the trapping sites for *Chrysomya marginalis,* suggests that this species does not have a strong habitat preference. *Chrysomya albiceps* had no spatial specialisations, which is probably due to it being a predatory species in the larval stage.

The site at the stables (site 1) was significantly different from the other sites (Table 2.1) for *Chrysomya chloropyga.* This suggests that *C. chloropyga* is attracted to horses or horse dung. Most of the other sites had either domestic animals or livestock and did not attract as many flies. Alternatively, the city dump was within 200m of the stables and this may also have contributed to the large numbers of this species, since it is abundant there (pers. obs.).

Like *C. chloropyga*, *C. putoria* showed a significant difference between the trapping sites. The two sites in Grahamstown East (sites 7 & 8) and a residential site (site 6) all peaked at much higher numbers than the other sites. This may be explained by the chickens in the Grahamstown East sites and geese at site 6, because *C. putoria* has been shown to breed in poultry manure (Hulley, 1983)

The trap at the stables (site 1) caught significantly more *Chrysomya megacephala* than the other traps. This may be due to the close proximity to the city dump, as Laurence (1988) observed *C. megacephala* breeding in large numbers in rubbish tips and septic tanks.

There is no clear spatial pattern in the Sarcophagids and this is probably because of the poor taxonomic resolution of the sample.

Summary

In the forensic context, the presence of puparia of species characteristic of particular seasons may have significance on corpses and carcasses that have passed active decay. The presence of certain human activities clearly affects the relative abundance of several blowfly species, which might be of relevance in some forensic investigations. The association of *Chrysomya putoria* with latrines, manure and excrement is an example.

Knowledge of the seasonal and spatial trends of these forensically important flies also has potential use in veterinary and public health issues. Sheep-strike is of particular interest in a veterinary context as *Lucilia* spp. and *Chrysomya chloropyga* (Smit & du Plessis, 1926; Mönnig & Cilliers, 1944) have been found to be primary flies in causing sheep-strike. Medicinal uses of flies for maggot therapy may also be affected by factors such as the lack of separation of *Lucilia sericata* and *Lucilia cuprina* into two distinct species.

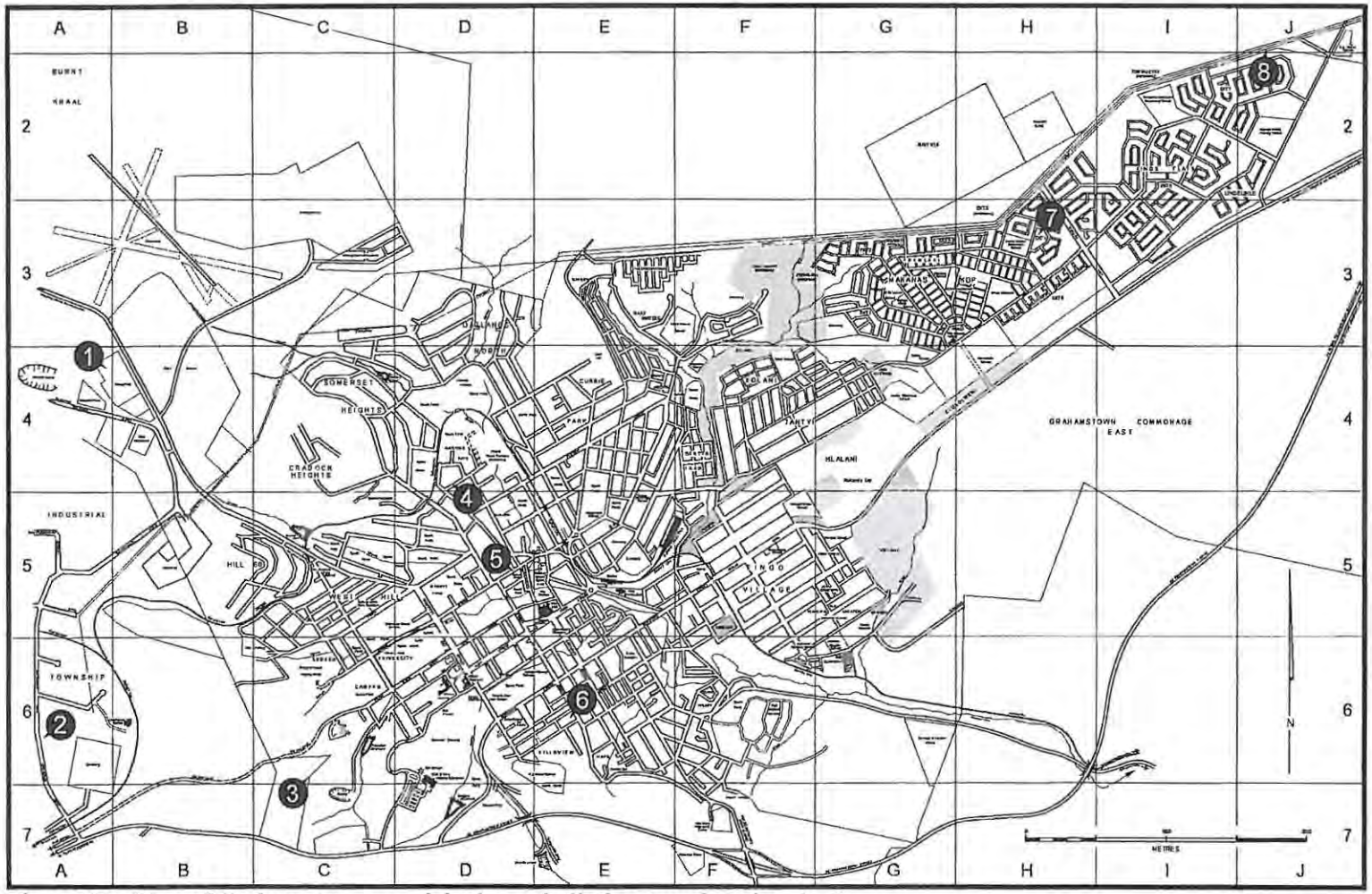

Figure 2.2: Map of Grahamstown municipal area indicting trapping sites 1 - 8

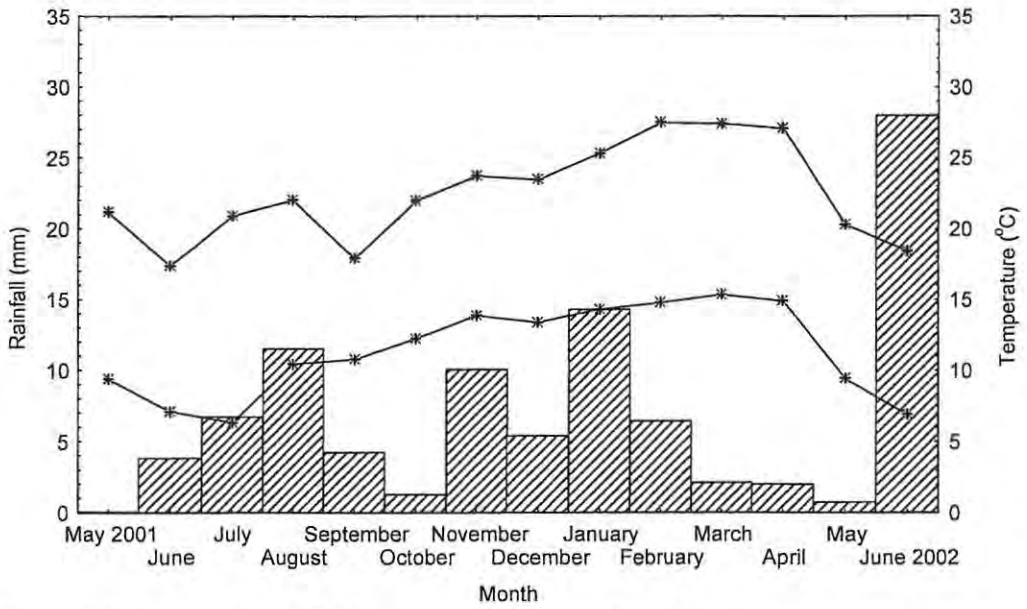

Figure 2.2: Average rainfall histogram and mean minimin and maximum temperatures for the trapping weeks in the Grahamstown municipal area from May 2001 to June 2002

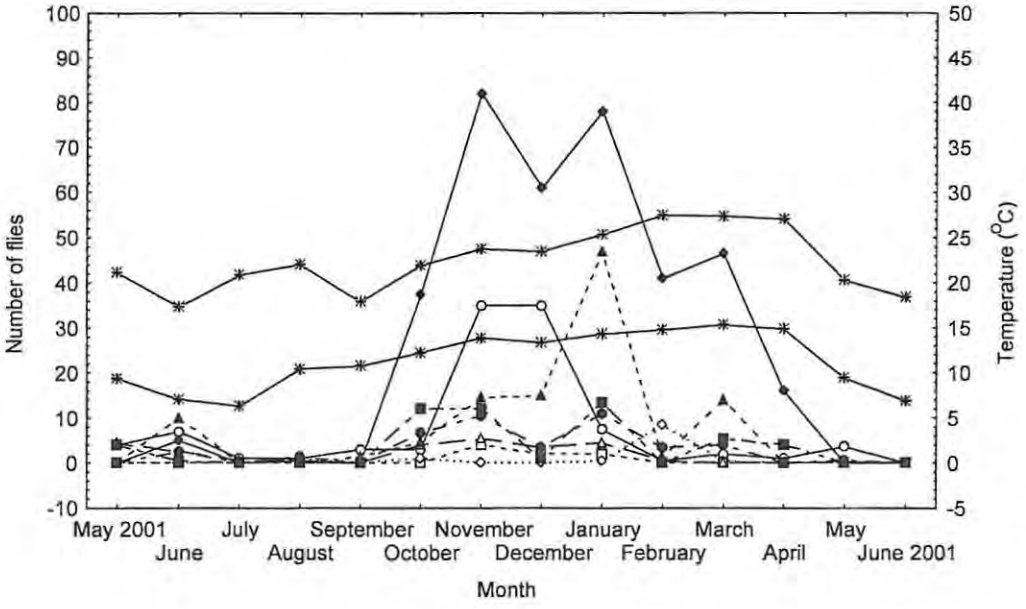

Figure 2.3: Seasonal distribution of *Lucilia sericata* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

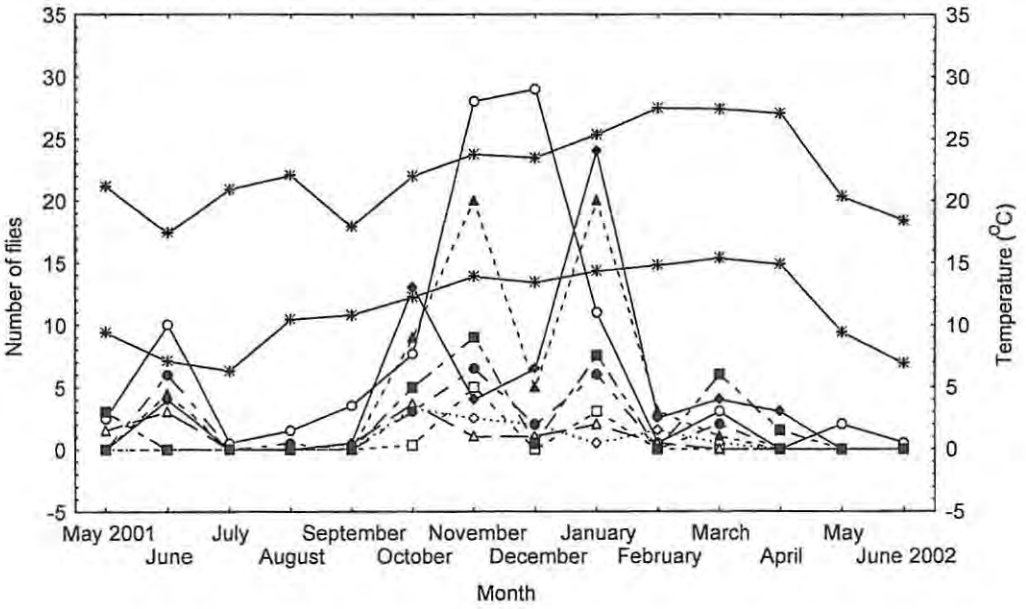

Figure 2.4: Seasonal distribution of *Lucilia cuprina* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

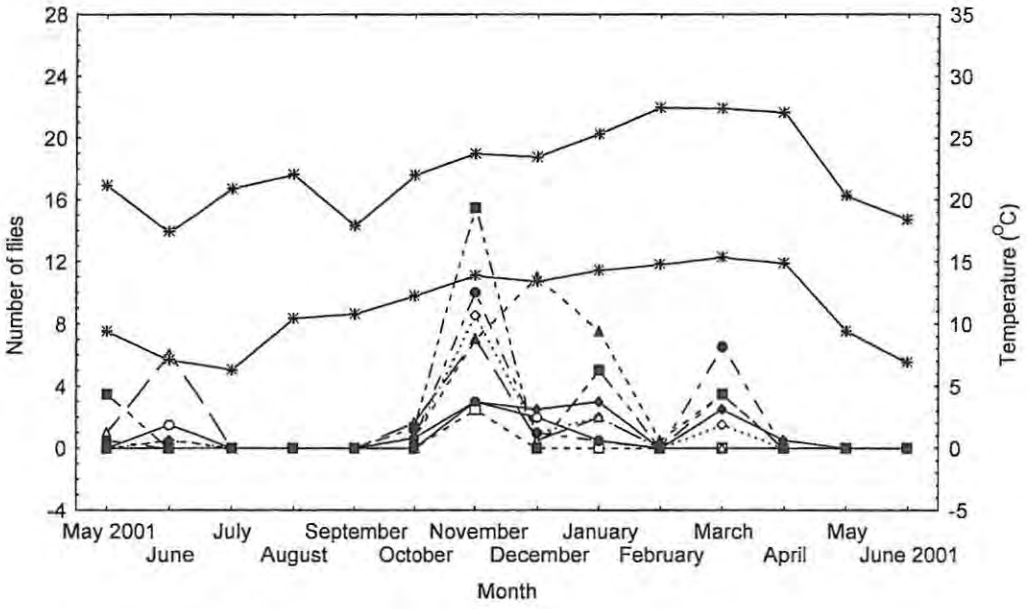

Figure 2.5: Seasonal distribution of *Chrysomya marginalis* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

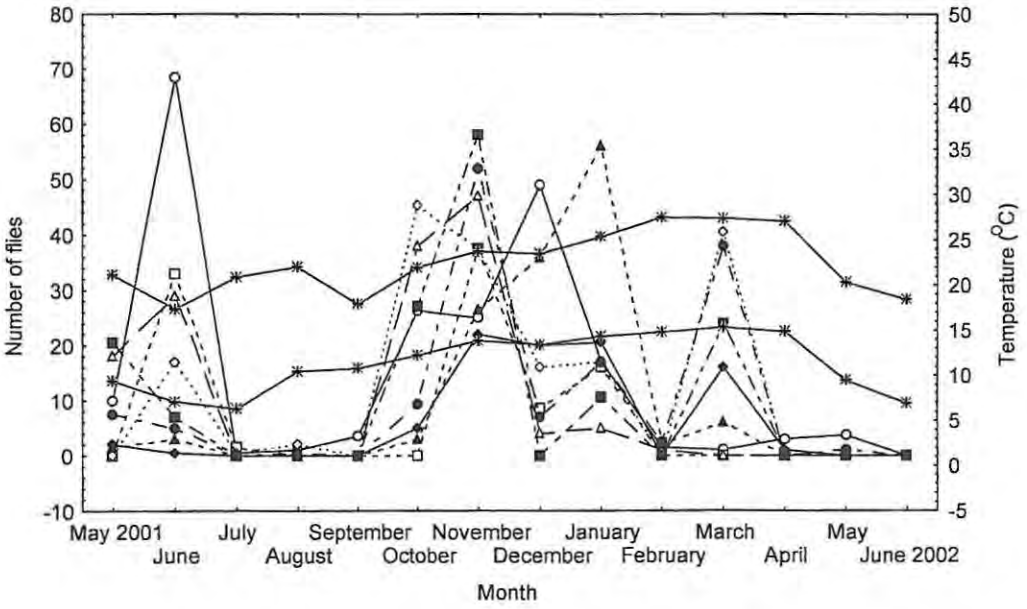

Figure 2.6: Seasonal distribution of *Chrysomya albiceps* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

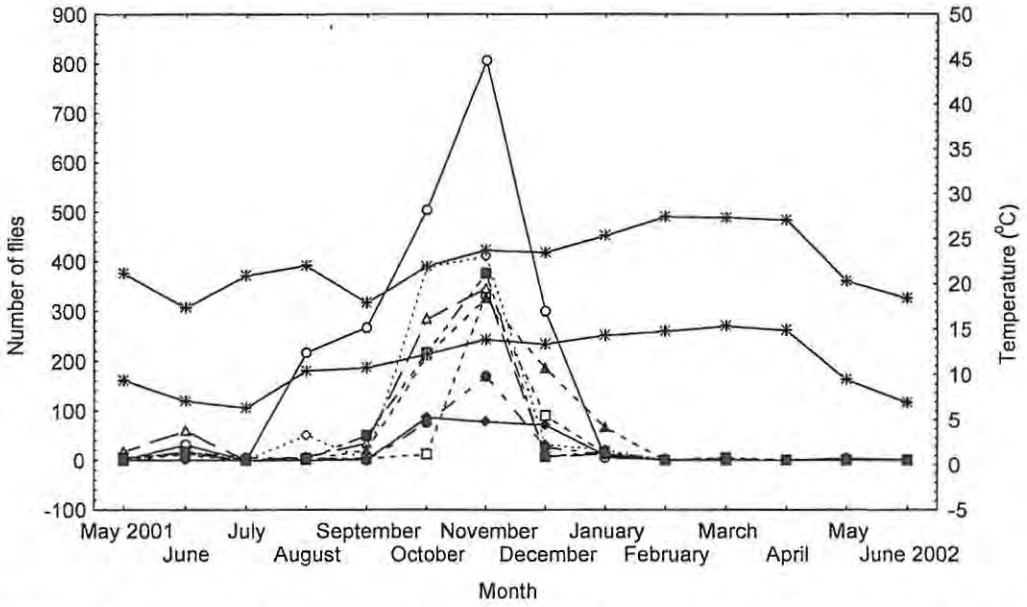

Figure 2.7: Seasonal distribution of *Chrysomya chloropyga* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

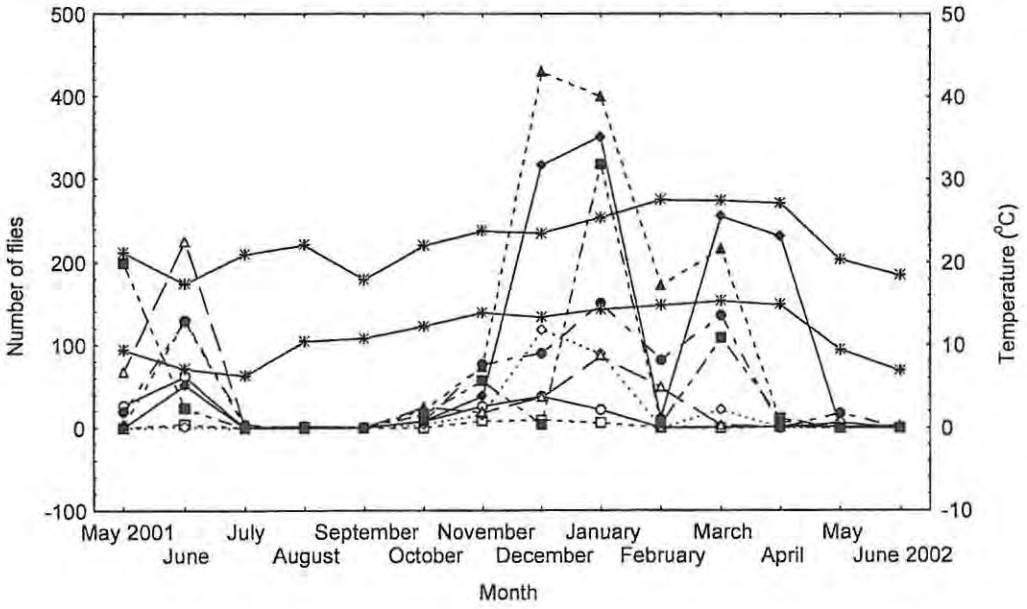

Figure 2.8: Seasonal distribution of *Chrysomya putoria* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

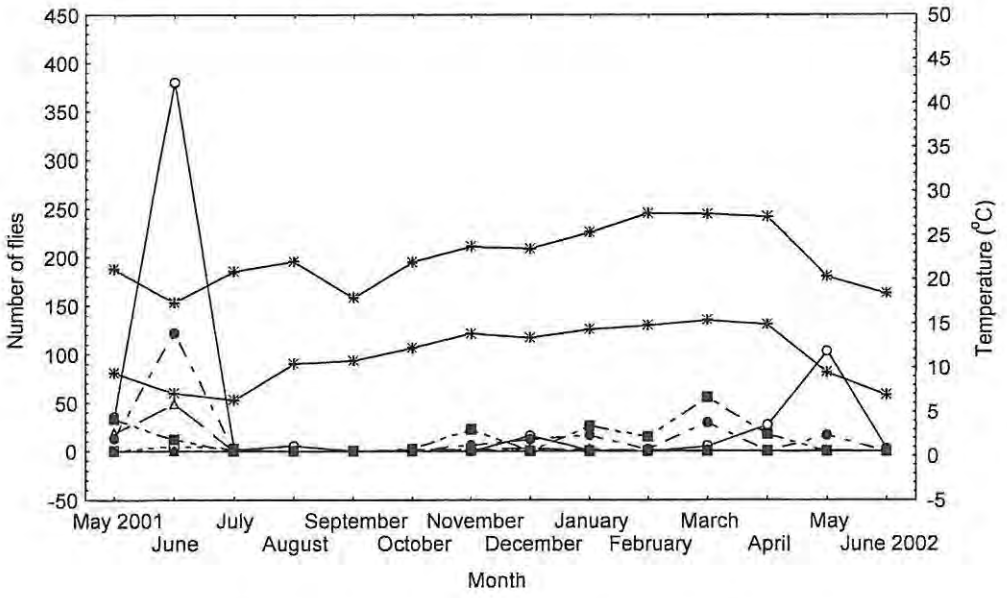

Figure 2.9: Seasonal distribution of *Chrysomya megacephala* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

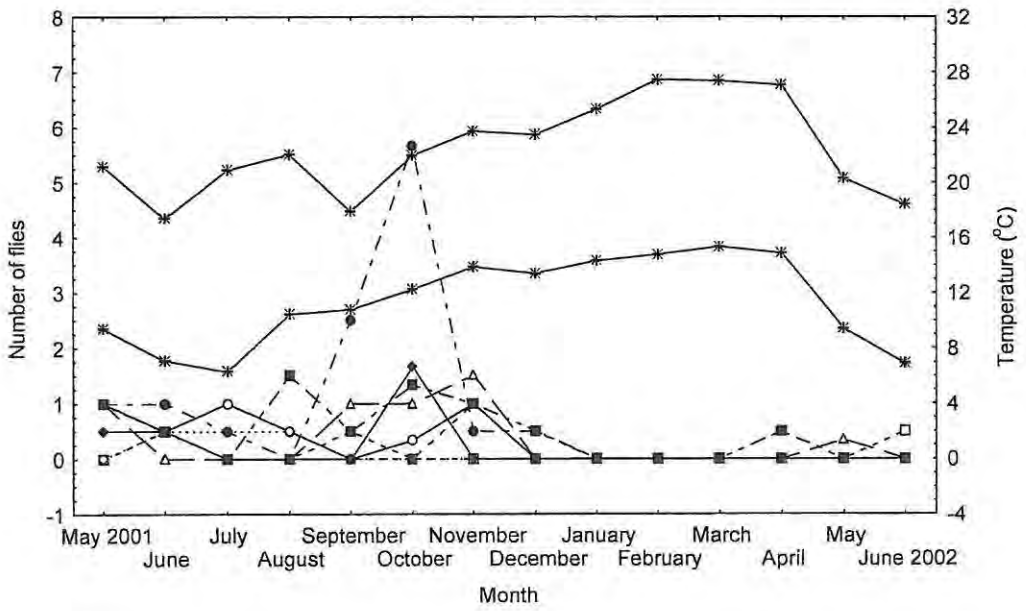

Figure 2.10: Seasonal distribution of *Calliphora croceipalpis* in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

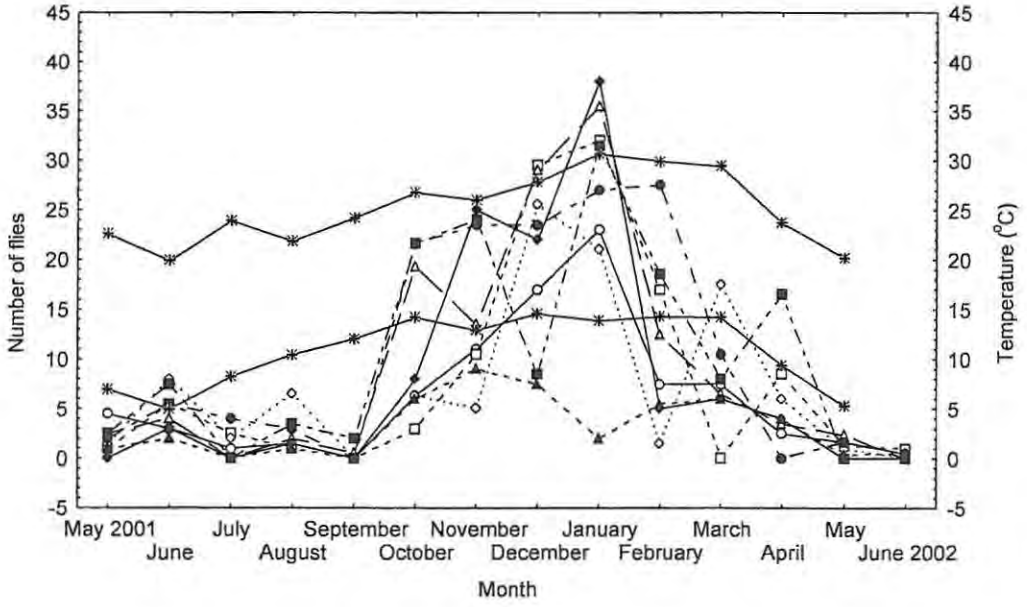

Figure 2.11: Seasonal distribution of *Sarcophaga* spp. in Grahamstown (trap 1 = ○, trap 2 = □, trap 3 = ◊, trap 4 = Δ, trap 5 = ●, trap 6 = ■, trap 7 = ♦, trap 8 = ▲, minimum/maximum temperature = *)

Table 2.1: Statistical values of ANOVA tests used in analysis of trapping data obtained from May 2001 to June 2002 in the Grahamstown area

Species	Month	Trap	Month*Trap
Lucilia sericata	F = 3.615	F = 8.427	F = 1.034
	P = 0.0000	P = 0.0000	P = 0.4272
Lucilia cuprina	F = 6.837	F = 5.619	F = 1.245
	P = 0.0000	P = 0.0000	P = 0.1255
Chrysomya marginalis	F = 7.031	F = 1.651	F = 0.787
	P = 0.0000	P = 0.1268	P = 0.8874
Chrysomya albiceps	F = 4.882	F = 0.489	F = 0.604
	P = 0.0000	P = 0.8412	P = 0.9944
Chrysomya chloropyga	F = 2.130	F = 5.768	F = 1,397
	P = 0.0000	P = 0.0000	P = 0.0406
Chrysomya putoria	F = 4.127	F = 3.195	F = 0.810
	P = 0.0000	P = 0.0038	P = 0.8571
Chrysomya megacephala	F = 2.123	F = 2.303	F = 1.021
	P = 0.0168	P = 0.0304	P = 0.4527
Calliphora croceipalpis	F = 1.724	F = 1.118	F = 0.596
	P = 0.0633	P = 0.3555	P = 0.9954
Sarcophaga spp.	F = 13.917	F = 2.203	F = 0.792
	P = 0.0000	P = 0.0381	P = 0.8804

Table 2.2: Periods of peak abundance of blowflies in various localities in South Africa

Species	Smit (1931)	Hepburn (1943a)	Mönnig & Cilliers (1944)	Zumpt & Patterson (1952)	Braack & de Vos (1987)
	Grootfontein	Onderstepoort	Bredarsdorp	Johannesburg	Kruger National Park
Lucilia spp.	Oct – Dec; April - May	July – Sept; Dec - March	Sept - Oct	Oct – Jan; March - April	June – Oct; Jan – March
Chrysomya chloropyga	Oct – Dec; March - May	Sept – Oct	Sept - Dec	Dec - Jan	July – Sept
C. albiceps	Nov – Jan; April - May	Oct – Dec; May	Dec - May	Dec - April	Nov – May
C. marginalis	March - May	Oct - Feb	Jan - April		Oct - Feb
C. putoria					Oct – March
Calliphora croceipalpis	Sept – Oct; March - June		Sept - Oct	June – Sept	

Table 2.3: Periods of peak abundance of blowflies in various localities in the world (NH = northern hemisphere)

Species	Fuller (1934)	Williams (1953)	Schoof & Savage (1955)	Murray (1956)	Linhares (1981)	Martinez-Sanchez *et al.*, (2000)
	Australia	New York	USA	New Zealand	Campinas City Brazil	Spain
Lucilia spp.	Oct - March	May – Oct (NH)	June – Aug (NH)	Summer months	Throughout year Aug peak	Aug – Sept (NH)
Chrysomya chloropyga					Aug – Oct; Jan – April	
C. albiceps					March – April; Aug	Aug – Sept (NH)
C. megacephala					Aug; April – July	

CHAPTER 3

Geographic distribution of forensically important flies in South Africa

INTRODUCTION

To make use of blowflies in forensic investigations, one needs to know what blowflies are found in the different regions of a country. The geographic occurrence of certain species, especially ectotherms such as insects, may be limited by physical factors such as temperature, rainfall and humidity.

Predictor models allow one to use known localities of a species to predict, on the basis of physical factors such as altitude, temperature, rainfall and humidity, where that species may occur (Robertson *et al.*, 2001). It is difficult to assess the absence of certain species in some areas as their absence may be due to seasonal occurrence, local extinction or lack of proper sampling (Robertson *et al.*, 2001). Thus presence records are more useful in prediction models.

The aim of this work was therefore to use known localities of forensically important blowflies from around South Africa and a predictive modelling technique to produce habitat suitability maps for each species.

MATERIALS AND METHODS

Museum records of the geographic occurrence of forensically important blowflies were obtained from the Natal Museum (Pietermaritzburg), South African Museum (Cape Town), the National Collection of Insects (Pretoria), the Albany Museum (Grahamstown) and the Rhodes University collection. Additional records were obtained from trapping surveys conducted in the Northern and Western Cape and Mpumalanga and from specimens collected at various localities in South Africa (Fig. 3.1).

Monthly average minimum and maximum temperatures, rainfall and humidity were used in a Principal Components Analysis (PCA) (Robertson *et al.*, 2001) to predict the

distribution of each species of forensically important South African blowflies. The technique constructs a hyperspace for the target species using principal component axes derived from a principal components analysis performed on the values of the predictor variables (temperature, rainfall, humidity) that are associated with the localities where the species has been recorded (Robertson *et al.*, 2001). These variables were chosen because they are the most important factors affecting the distribution of blowflies (Smith, 1986; Chapters 2 & 5).

The following numbers of distinct locality records for each species were used in each PCA: *Lucilia sericata* – 48; *L. cuprina* – 35; *Chrysomya marginalis* – 82; *C. albiceps* - 78; *C. chloropyga* – 101; *C. putoria* – 31; *Calliphora croceipalpis* – 44. There were too few records for *Chrysomya megacephala* to perform a meaningful prediction.

RESULTS

The prediction maps (Figs. 3.2 – 3.8) indicate areas of climatic suitability for each species based on temperature, humidity and rainfall from the areas in which they have been recorded. The maps were divided evenly into 5 classes, with the darker areas indicating areas of higher suitability.

Lucilia sericata was recorded from in most areas of South Africa with few records from the Northern Cape, Northern Province and KwaZulu Natal. The PCA model indicated high suitability for this species in the Eastern and Western Cape and large parts of central South Africa (Fig. 3.2).

Lucilia cuprina was also recorded throughout South Africa with few records from the Northern Cape, Northern Province and KwaZulu Natal. The PCA model indicated high suitability for this species in the Eastern Cape going into KwaZulu Natal and central South Africa through the Free State, Northern Cape and into Gauteng and Mpumalanga (Fig. 3.3).

Chrysomya marginalis was recorded throughout South Africa. The PCA model indicated high suitability for this species throughout South Africa with the exception

of the western part of the Western Cape, which is a winter rainfall area, and the Lesotho highlands (Fig. 3.4).

Chrysomya albiceps was recorded in most parts of South Africa with the exception of KwaZulu-Natal. The PCA model indicated high suitability for this species in all regions excluding Limpopo and the western part of the Western Cape (Fig. 3.5).

Chrysomya chloropyga was recorded throughout South Africa with fewer records in the Northern Cape. The PCA model indicated high suitability for this species throughout South Africa with reduced probability in the north of the Northern Cape (Fig. 3.6).

Chrysomya putoria was recorded largely along the coastal regions and in the northern parts of South Africa. The PCA model indicated high suitability for this species along the eastern coast of South Africa and up into Mpumalanga and Limpopo (Fig. 3.7).

Calliphora croceipalpis was recorded in the Western and Eastern Cape, KwaZulu Natal and North West province. The PCA model indicated high suitability for this species in the Western Cape along the coastal areas, the Eastern Cape up into the Free State, Mpumalanga, Gauteng and the Limpopo valley (Fig. 3.8).

DISCUSSION

The predicted distributions indicate that with minor exceptions, the whole of South Africa is suitable for the existence of all the forensically important species of blowfly (Fig. 3.1 – 3.8).

The predictions for *Lucilia sericata* and *L. cuprina* (Figs. 3.2 & 3.3) agree with comments by Braack & De Vos (1987) who observed that *L. cuprina* was found in much larger numbers in the Kruger National Park (eastern Mpumalanga and Limpopo) than *L. sericata*. *Lucilia cuprina* is known as the sheep-strike fly and its widely predicted area of suitability suggests that this is why it has been and continues to be, such a veterinary problem (Smit, 1931; Hepburn, 1943a,b; Mönnig & Cilliers, 1944). *Lucilia sericata* was introduced from Europe and should therefore show

adaptations to colder weather. Its low suitability for KwaZulu Natal agrees with this as the eastern side of South Africa experiences more tropical weather. Both of these species are widely distributed and therefore are likely to be found at most crime scenes.

Chrysomya marginalis was collected throughout South Africa and the predictions agree with this (Fig.3.4). The small area in the Western Cape where low suitability is indicated may be as a result of few records from this region rather than lack of suitability as this species was recorded from this area. As this species is predicted to occur to such a large extent in the rest of the country, it seems unlikely that it will not occur in the Western Cape. This species is likely to be important at murder scenes throughout South Africa.

Chrysomya albiceps is widely distributed and is predicted to occur in most parts of South Africa (Fig. 3.5). This species is a facultative predator that feeds on other larvae (Prins, 1982; Braack & de Vos, 1987) and is likely to be present at most corpses that are decaying.

Chrysomya chloropyga occurs along the coastal regions and in the northern parts of South Africa. The lack of suitability for this species in the Northern Cape (Fig. 3.6) is likely to be partly due to seasonal variations, as this area was sampled late in summer when this species does not occur in large numbers. Thus the prediction of suitability for this species in the Northern Cape may increase if more sampling is undertaken at a different time of year. Despite this, *C. chloropyga* is still likely to occur at least at low densities in most parts of South Africa and will therefore be of forensic importance.

Chrysomya putoria's highest suitability predictions are largely on the eastern side of South Africa (Fig. 3.7). Very few locality records (31) were available for this species and as a result, the restricted suitability may be partly attributed to lack of sampling. However, this species is reported further north in Africa (Smit, 1931; Paterson, 1968; Pont, 1980) where it is warmer and thus the eastern part of South Africa, which is more tropical, is likely to be a more suitable habitat (Paterson, 1968). This species' forensic importance might be limited, especially in light of its high preference for poultry manure (Hulley, 1983; Chapter 2).

Calliphora croceipalpis is predicted to occur along the Southern coast and further north from the Eastern Cape (Fig 3.8). Its occurrence has been restricted to the coastal regions and some inland areas in the Eastern Cape and Free State. This may be due to it being a winter and early spring fly (Chapter 2) and it thus prefers the higher-lying areas that are colder in winter and the coastal regions that experience winter rainfall. This species is therefore most likely to occur at crime scenes in the cooler parts of the country.

The PCA method used for producing the prediction maps is a useful tool in forensic entomology for predicting what forensically important species are likely to be found in different parts of a country. The more presence records available, the more reliable the prediction maps will be (Robertson, pers. comm.). As blowflies are able to travel 2-3km/day (Braack & Retief, 1986), they are likely to reach most areas of South Africa that are suitable based on the temperature, humidity and rainfall. Insects that are not able to disperse as rapidly are therefore likely to be restricted to areas based on geographic features. This was clearly shown in silphids (Robertson & Villet, submitted) where two species occurred on opposite sides of the country and only overlapped in a small area due to difference in habitat preferences. More forensically important species should be modelled to make more use of forensic entomology in South Africa.

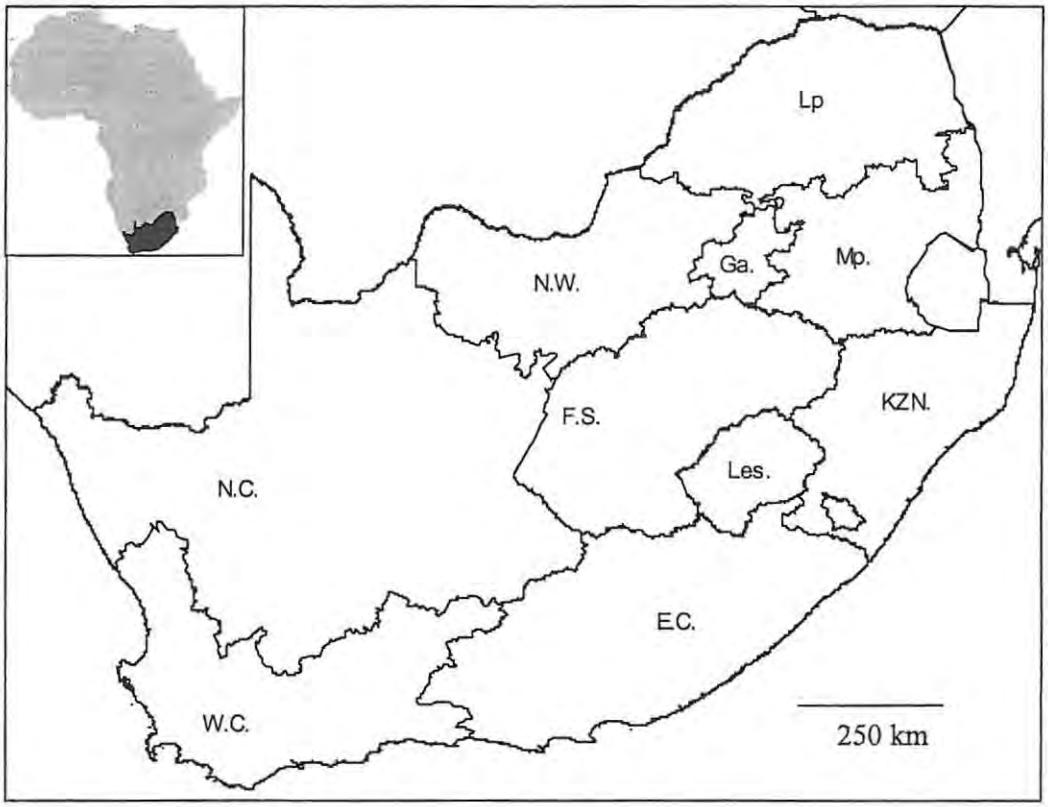

Figure 3.1: Map of South Africa indicating the provinces. The inset indicates South Africa relative to Africa. EC = Eastern Cape; FS = Free State; Ga = Gauteng; KZN = KwaZulu Natal; Les = Lesotho; Lp = Limpopo; Mp = Mpumalanga; NC = Northern Cape; NW = North West; WC = Western Cape.

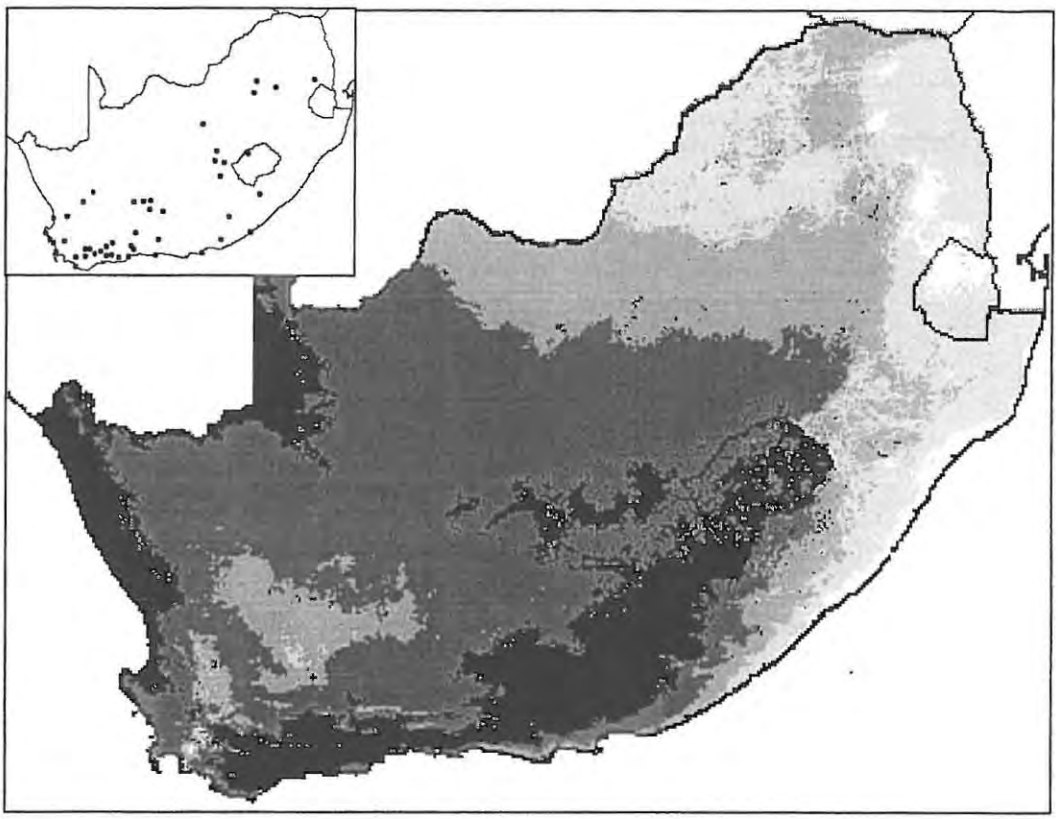

Figure 3.2: Bioclimatic suitability map for *Lucilia sericata* in South Africa produced from 48 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

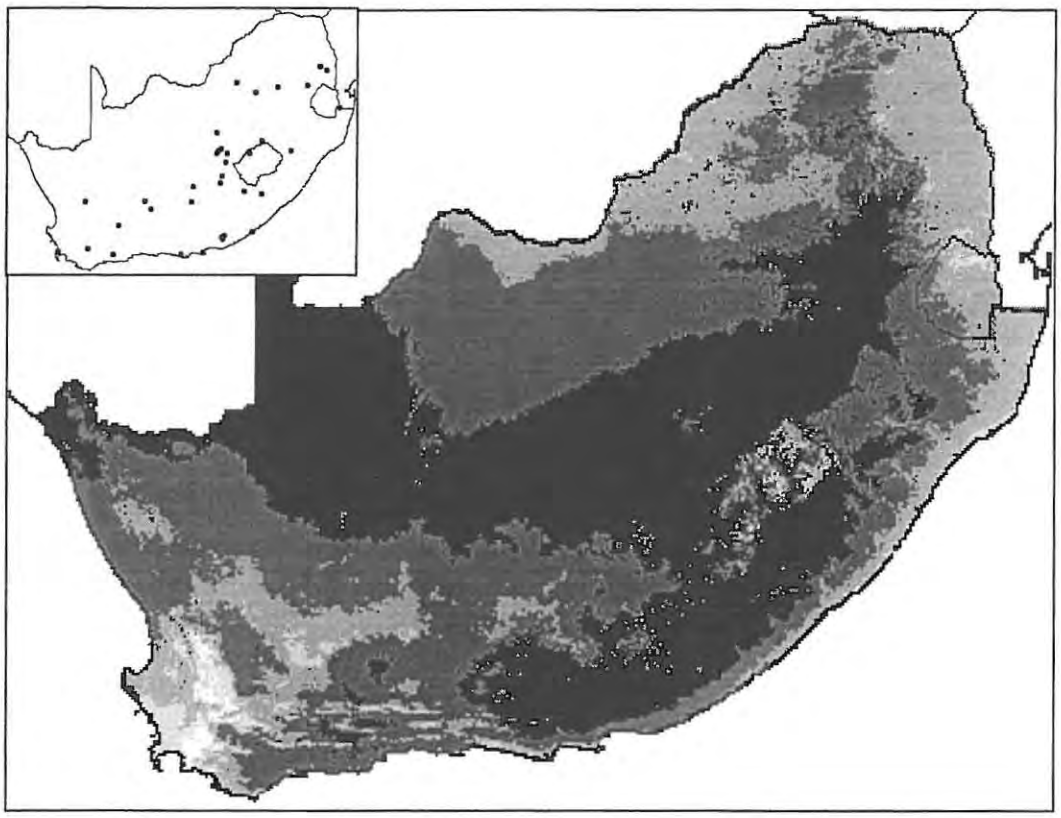

Figure 3.3: Bioclimatic suitability map for *Lucilia cuprina* in South Africa produced from 35 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

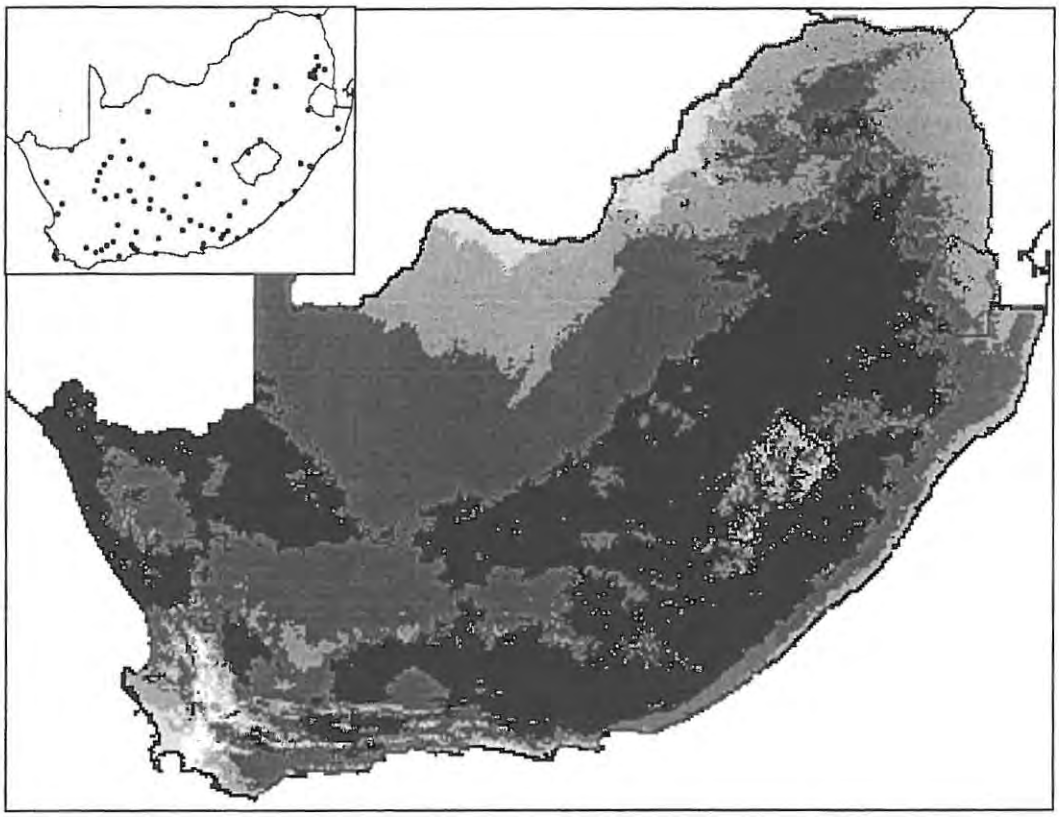

Figure 3.4: Bioclimatic suitability of *Chrysomya marginalis* in South Africa produced from 82 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

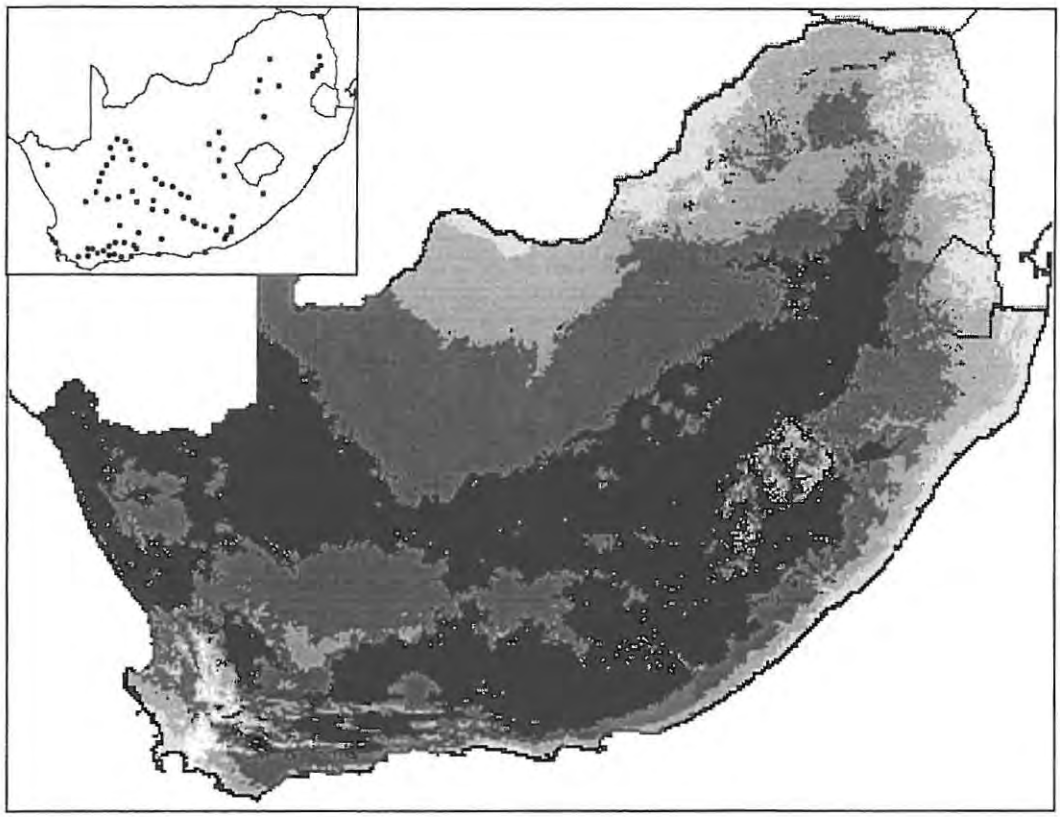

Figure 3.5: Bioclimatic suitability map for *Chrysomya albiceps* in South Africa produced from 78 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

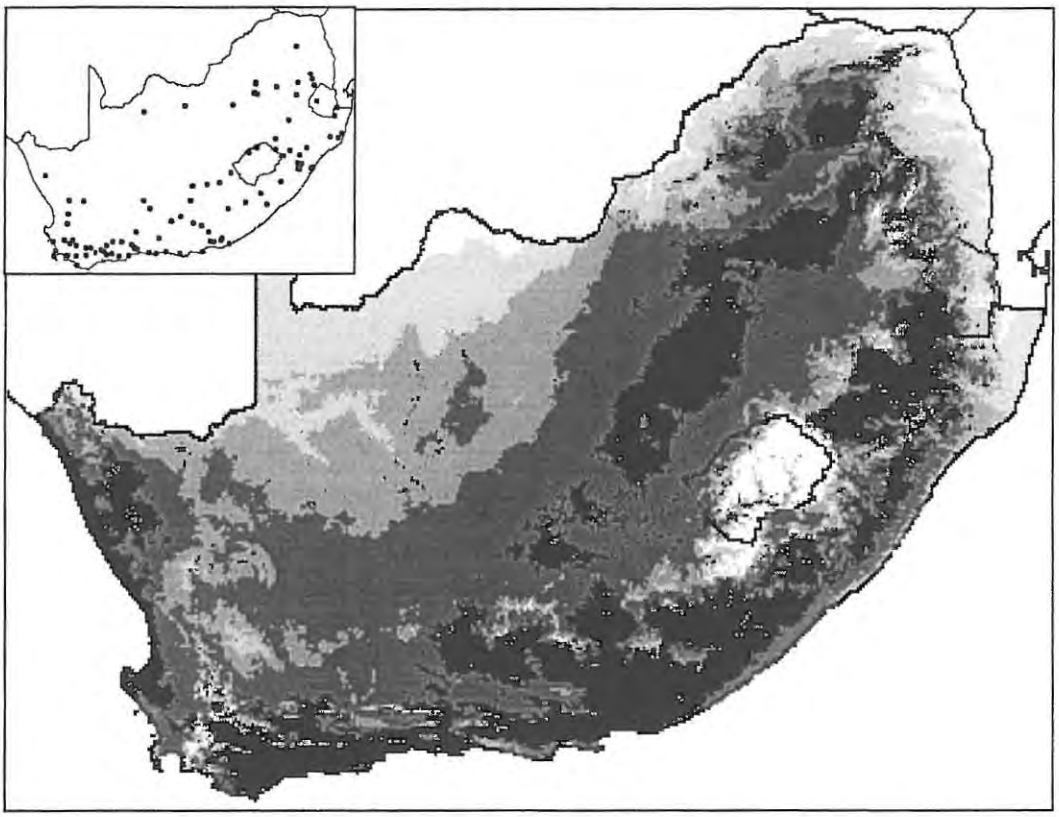

Figure 3.6: Bioclimatic suitability for *Chrysomya chloropyga* in South Africa produced from 101 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

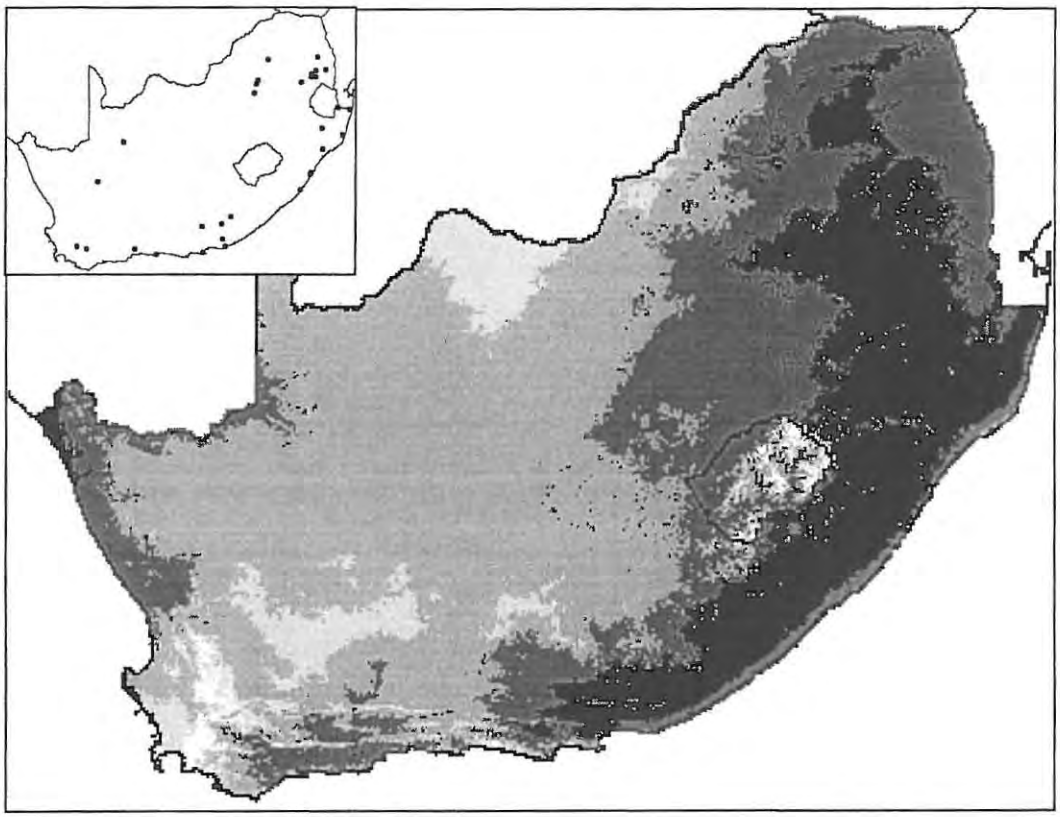

Figure 3.7: Bioclimatic suitability map for *Chrysomya putoria* in South Africa produced from 31 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

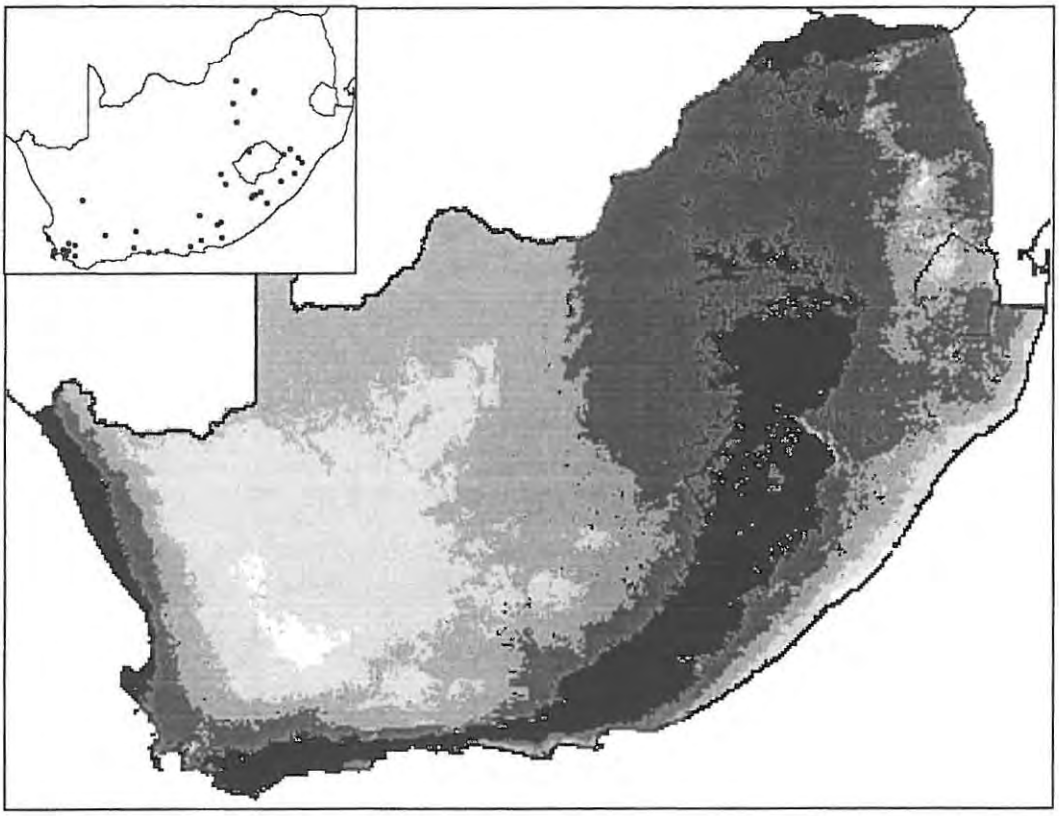

Figure 3.8: Bioclimatic suitability map for *Calliphora croceipalpis* in South Africa produced from 44 localities (see inset) where the species was recorded. Darker shades indicate higher probabilities.

CHAPTER 4

Nocturnal oviposition in forensically important flies: laboratory and field studies

INTRODUCTION

In the past it was generally accepted that blowflies were not active at night and therefore did not oviposit in the dark (Greenberg, 1985). This assumption could affect the estimation of post mortem intervals (PMI), particularly since many unattended deaths occur at night. However, Green (1951) reported *Calliphora* flying at night and ovipositing small egg batches on livers and cut surfaces of carcasses in British slaughterhouses. Greenberg (1990) and Singh & Bharti (2001) have also shown *Calliphora vicina* Roineau-Desvoidy, 1830, *Lucilia sericata*, *Phormia regina* (Meigen, 1826) *Chrysomya megacephala* and *C. rufifacies* (Macquart) to be active at night, ovipositing on carcasses and mutton pieces. This fact is potentially important in estimating post mortem intervals (PMI) as nocturnal oviposition can alter the precision of a PMI estimate by up to 12 hours (Greenberg, 1990).

While nocturnal oviposition is possible in five species, of four different genera, there is a need to establish the generality of this result, and to extend it specifically to species of concern in Southern Africa. *L. sericata* and *C. megacephala* are found in South Africa, so this work allows direct comparisons to previous studies. South Africa is also geographically distant from and distinct from the previous study sites (Greenberg, 1990; Singh & Bharti, 2001).

MATERIALS AND METHODS

Two experiments were conducted, one field-based and the other laboratory-based.

Field Experiments

The field experiment was carried out on the flat roof of the Biological Sciences building at Rhodes University. It was conducted in November 2001 and again in February 2002. Four days in each month were used for the experiment and these coincided with four benchmarks in the lunar cycle: full moon, first quarter, last

quarter and new moon. Rats that had been dissected were collected, placed in plastic bags and frozen. Twelve rats were thawed two days prior to each day of experiment. On the day of experiment, the plastic bag containing the rat was opened and placed in a 2l plastic container. The container was placed on top of the wall surrounding the roof and left for 2 hours, starting at 10am each day. The rat was replaced every 2 hours for a 24-hour period. Light intensity readings were taken at night using a Gossen Lunasix 3 photographic light meter. After exposure, each rat was placed in a constant environment (CE) room at 20°C to allow any larvae/eggs to complete their development. The containers were covered with netting secured with an elastic band. Any flies that emerged were left in the container and identified when dead.

Laboratory Experiments

The laboratory experiment was conducted in two CE rooms at 20°C. One was set at normal photoperiod and the second at reversed photoperiod of 12h:12h, so that day and night would be experienced by two sets of flies during normal working hours and thus make it simpler to record the flies' activity in the dark.

Forty flies of each species were used for each replicate. Twenty flies were placed in each of two holding cages and one cage was placed in a CE room at normal photoperiod while the other was placed in a CE room at reversed photoperiod. The flies were provided with milk powder, sugar and water *ad libitum*. They were allowed to acclimate for 4 days before the experiment was conducted. On day 1 of the experiment, each female fly was put into its own 1-litre bottle containing a piece of pork and cotton wool soaked in sugar water. The bottle mouth was closed with netting secured with an elastic band. Ten bottles for each species were set up in each CE room. The bottles were checked periodically through the day for eggs. The bottles were specifically checked before the lighting change every day. A red light was used to check the bottles in the dark so as to not disturb the flies and was left on between checks for the same reason. The experiment ran for 3 days, and 3 replicates were carried out for each species. This experiment was conducted using *Lucilia* spp; {*L. sericata* and *L. cuprina* were combined because interbreeding and possible hybrids have been observed (Ullyett, 1945)}; *Chrysomya chloropyga*, *C. putoria* and *C. megacephala*.

RESULTS

There was only one night-time laying, by *Lucilia* spp., during the two months that the field experiment was conducted. This occurred on 20th February 2002, which coincided with the last quarter of the moon that rises at midnight. It was also the only calliphorid that laid eggs throughout the experiment. Sarcophagid species were commonly attracted to the rats during the day, laying on all of the days, except on one when the weather was inclement. The temperature at which the night-time laying occurred was 19.7°C, while the daytime laying occurred at temperatures between 21°C and 36°C (Table 4.1). No obvious bias towards a specific time of day was noted in the daytime laying.

Lucilia spp., *C. putoria* and *C. chloropyga* laid once at "night" in the laboratory experiment (Table 4.2). *C. megacephala* did not lay at "night" and only one female laid eggs in the entire 9-day experiment. Nine batches of eggs were laid by *Lucilia* spp., eight by *C. putoria* and three by *C. chloropyga*.

DISCUSSION

Previous studies on nocturnal oviposition (Greenberg, 1990; Singh & Bharti, 2001) suggest that calliphorids lay eggs at night in approximately one in three carcasses. This is a relatively high percentage and potentially misleading. In this study only one night-time oviposition event took place out of seven nights. This is substantially lower than pervious studies (Greenberg, 1990; Singh & Bharti, 2001) and implies that caution must be taken when looking at the possibility of nocturnal oviposition. There is little doubt that nocturnal oviposition occurs, as this study and previous ones (Greenberg, 1990; Singh & Bharti, 2001) have shown. However, the frequency of these events may vary greatly with season, temperature, geographic location and species. If oviposition occurs at night, this can alter a PMI by up to 12 hours. Thus forensic entomologists need to bear in mind that although nocturnal oviposition occurs infrequently, it is a possibility.

The sizes of carcasses used in experiments on nocturnal oviposition have all been relatively small. Greenberg (1990) and this study used rats, while Singh & Bharti (2001) used pieces of mutton. Denno & Cothran (1975) showed that different size

carcasses attract different flies. In their field studies using rat and rabbit carcasses, the former were dominated by sarcophagids (64%) and only 29,3% by *Lucilia sericata*. The rabbit carcasses were less attractive to sarcophagids (2,8%) while *L. sericata* was still attracted at the same rate (29,3%). In experiments conducted by Mönnig & Cilliers (1944), carcasses under 500g attracted different blowflies to larger carcasses. Only *L. sericata*, *Calliphora croceipalpis* and *Sarcophaga* spp. were recorded as breeding in small carcasses. *L. sericata* is reported to breed mainly in small carcasses and is active throughout the year, while *Chrysomya* spp. appear to prefer larger carcasses (Mönnig & Cilliers, 1944; Ullyett, 1950; Braack, 1987).

Meskin (1980) found the same trend of *L. sericata*, *C. croceipalpis* and *Sarcophaga* spp. breeding readily in rats and small bird carcasses and sarcophagids breeding in snails. He thus suggested that because sarcophagids deposit larvae, they are able to make better use of small carcasses that dry out very rapidly. This study found this phenomenon with sarcophagids depositing larvae in the rats in consistent numbers during the day. Therefore, the use of small carcasses in the study of nocturnal oviposition may introduce bias and future studies with larger carcasses would be advised.

The use of carcasses that have been frozen and then thawed may also affect the natural sequence of decomposition and associated emission of volatile attractants (Micozzi, 1986). It would be advisable to use fresh and frozen-thawed carcasses where possible to compare possible differences in the decomposition sequence.

The results of the laboratory experiment show *Lucilia* spp., *Chrysomya putoria* and *C. chloropyga* capable of laying in complete darkness. This suggests that these species will lay in carcasses at night if the ideal circumstances arise. Green (1951) found *Calliphora vicina*, *L. sericata* and *Phormia terraenovae* laying in complete darkness in laboratory experiments. Temperature appears to be the most important limiting factor. *C. megacephala* did not lay at night, although Singh & Bharti (2001) recorded this species laying 3 times at night in their nocturnal oviposition experiments. The use of larger pieces of meat/carcasses may yield greater success.

There does not appear to be any correlation between the moon phase and the night-time laying (Table 4.1). The only night-time oviposition occurred at last quarter which emits less light than full moon and rises at midnight. In the laboratory experiments the "night-time" oviposition was in complete darkness and thus light does not appear to be a necessary factor for oviposition to occur.

Table 4.1: Conditions associated with oviposition events on rats over a 24h period outdoors. (N/A = not applicable because lux > 10)

Species	Date	Time	Average Temperature (°C)	Lux	Lunar Phase
Sarcophaga spp.	1/11/2001	10:00 – 14:00	26.9	N/A	Full Moon
Sarcophaga spp.	22/11/2001	10:00 – 18:00	24.6	N/A	Last Quarter
	23/11/2001	8:00 – 10:00			
Sarcophaga spp.	5/2/2002	10:00 –12:00	26.2	N/A	First Quarter
Sarcophaga spp.	13/2/2002	12:00 – 16:00	28.0	N/A	New Moon
Sarcophaga spp.	20/2/2002	10:00 –12:00	29.9	N/A	Last Quarter
		14:00 – 16:00			
Lucilia spp.	20/2/2002	22:00 –00:00	19.7	0.79	Last Quarter
Sarcophaga spp.	27/2/2002	10:00 –12:00	30.7	N/A	Full Moon
		14:00 – 18:00			

Table 4.2: Oviposition events by four blowfly species on pork in the laboratory.

Species	Day	Night	Total
Lucilia spp.	8	1	9
Chrysomya putoria	7	1	8
Chrysomya chloropyga	2	1	3
Chrysomya megacephala	1	0	1
Total	18	3	21

CHAPTER 5

Thermophysiological thresholds of adult flies in relation to forensic entomology

INTRODUCTION

The use of flies in forensic entomology to determine post mortem intervals (PMI) relies on the flies being present and active. Under certain conditions such as extreme heat or cold, flies may not be active.

Insects have a wide range of physiological responses to temperature. These include changes in metabolism, changes in respiration and effects on their nervous and endocrine systems (Neven, 2000). Lethal temperature limits are the physiological tolerance limits for an organism beyond which recovery is impossible. There has been a tendency to replace these with critical thermal maxima and minima as these studies take less time and are easier to conduct (Mitchell *et al.*, 1993). The critical thermal maximum has been studied in numerous insects (Mitchell *et al.*, 1993; Kharboutli & Mack, 1993). Numerous studies have been conducted on the effects of supercooling, cold storage and thermotolerance and the expression of heat shock proteins in flies (Davidson, 1969; Ring, 1972; Yocum & Denlinger, 1992, 1994; Leopold *et al.*, 1998), but little work has been conducted to determine the range of temperatures over which blowflies can survive.

This study was therefore designed to measure the temperature range in which adult blowflies are active and to relate this to their daily and seasonal distribution, which would affect their use in a forensic context.

MATERIALS AND METHODS

Twenty flies each of *Lucilia* species (*Lucilia sericata* and *L. cuprina* were grouped together because Ullyett (1945), cast doubts on the identity of these species by suggesting that they interbreed), *Chrysomya megacephala, C. chloropyga* and *Calliphora croceipalpis* were used for this experiment. The flies were chilled until immobile in an insulated container filled with ice-bricks. Once immobile, one fly at a

time was removed from the container and secured to a temperature probe (MT-29/1 hypodermic needle microprobe) stabbed off-centre through its thorax. The probe was attached to a Model BAT-12 digital thermometer. This method was similar to the one used on ants in Australia (Christian & Morton, 1992) and on cicadas in the USA (Sanborn & Phillips, 1996) to measure body temperature. The fly was then allowed to warm up while an infrared lamp (Maxamatic CT) was shone on it from a distance of 40cm and the following measurements were taken: temperature of first movement; temperature of walking; temperature of shade-seeking activity (temperature at which thermoregulation takes precedence over other behaviours: Sanborn & Phillips, 1996) and temperature of death. The fly was then weighed using a microbalance (BEL Mark 120A balance).

The results were analysed using ANOVA and Tukey's post-hoc pairwise comparisons to identify sources of significant differences in STATISTICA v5.

RESULTS

Except in *C. megacephala*, there was a statistically significant difference between the sizes of the male and female flies, with females being significantly larger in *Lucilia* spp, *Chrysomya chloropyga* and *Calliphora croceipalpis* (Table 5.1).

Calliphora croceipalpis had the lowest movement threshold, being able to move at an average of 14.53°C while *C. chloropyga* had the highest death threshold at an average of 52.64°C (Fig. 5.1).

The temperature at which movement occurred was statistically significantly different between *C. chloropyga* and *C. megacephala* (P = 0.02), *C. chloropyga* and *Lucilia* spp. (P = 0.03), *C. chloropyga* and *Calliphora croceipalpis* (P = 0.02) and between *C. croceipalpis* and *C. megacephala* (P = 0.03). All other comparisons were not significant.

At walking temperature *C. chloropyga* was statistically different from *C. megacephala* (P = 0.01). All other comparisons were not significant.

At shade-seeking temperatures, *C. megacephala* was statistically significantly different from *C. chloropyga* (P = 0.00), *Lucilia* spp. (P = 0.00) and *C. croceipalpis* (P = 0.00). *Calliphora croceipalpis* was also significantly different from *Lucilia* spp. (P = 0.00) and *C. chloropyga* (P = 0.03). All other comparisons were not significant.

Temperature at time of death differed significantly between some of the species. *C. megacephala* was significantly different to *Lucilia* spp. (P = 0.00) and *C. croceipalpis* (P = 0.00); *Lucilia* spp. was significantly different to *C. chloropyga* (P = 0.00) and *C. chloropyga* was significantly different to *C. croceipalpis* (P = 0.00). All other comparisons were not significant.

DISCUSSION

A clear sexual size dimorphism is evident in *Chrysomya chloropyga*, *Calliphora croceipalpis* and *Lucilia* spp. This may be to enhance fecundity because the larger the female, the more eggs she is able to lay (Ullyett, 1950). However, there was no uniform response to higher temperatures despite the females being larger than the males. In *Lucilia* spp. the males had slightly higher thermophysiological thresholds than the females. The thresholds of *C. chloropyga* were very similar between the sexes and in *C. croceipalpis* the females had a greater ability to survive the extreme temperatures than the males (Fig 5.1). *C. megacephala*, *C. chloropyga* and *C. croceipalpis* all followed a similar trend at the lower temperature, with the males being able to move at slightly lower temperature than the females. Only in *Lucilia* spp. did the females have a lower movement threshold than the males. Thus, despite a sexual size dimorphism, there appears to be no advantage in terms of surviving extreme temperatures.

C. megacephala had the highest shade-seeking temperature tolerance level of all the flies used in this experiment (Fig 5.1). This is unexpected as *C. megacephala* is active during the cooler winter months (Chapter 2). One would expect it to deal poorly with such high temperatures as it is unlikely to be exposed to such conditions in winter. It may be active in winter because it cannot compete with the flies that are found in summer, but would otherwise be common then as it is found in India and the Far East where it is very hot (Prins, 1979).

Lucilia spp. have a lower shade-seeking temperature tolerance than *C. megacephala*, but have a range of approximately 20°C between walking and shade-seeking (Fig 5.1). These flies are found year round with greater numbers in summer (Chapter 2). This thermal range would therefore allow them to be active year round and to live in most places in South Africa. But *L. sericata* prefers colder, mountainous areas (Chapter 3) and *L. cuprina* is more likely to occur in the interior (Chapter 3).

C. chloropyga is active in spring and early summer (Chapter 2). The very high death temperature recorded is therefore probably adaptive as there is a large range between the shade-seeking and death temperatures. This species is likely to use behavioural thermoregulation, moving out of the sun when it gets too hot to avoid warming the body to higher temperatures. As the ambient temperature is not likely to rise about 40°C in the shade very often, this species would be capable of being active in the shade for the entire day, as Nuorteva (1959) suggests that blowflies are active during sunshine and on cloudy days when the ambiente temperature is 16°C or higher.

Calliphora croceipalpis had the lowest thresholds of all the species in this experiment for all four measured points (Fig 5.1). This is probably because this species is most active in late winter into spring (Chapter 2). It does not have to deal with high ambient temperatures and is able to start moving at lower temperatures than the other species in this experiment (Fig 5.1). The lower shade-seeking temperature shows that this species dislikes high temperatures. It seems to prefer high ground and wet winters (Chapter 3). They are commonly found indoors and most of this species relatives are from Europe (Hall,1948; Erzinclioğlu, 1983).

All the species used in this experiment were able to move at temperatures below 20° and above 15°C (Fig 5.1). This suggests that these species could be active at night in summer when the ambient temperatures are above 15°C (Fig 5.1). Although only *Lucilia* spp. were found to lay at night (Chapter 4), the possibility exists that other

species are capable of being active at night and ovipositing. This has implications for PMI estimates and must be taken into consideration (see Chapter 4).

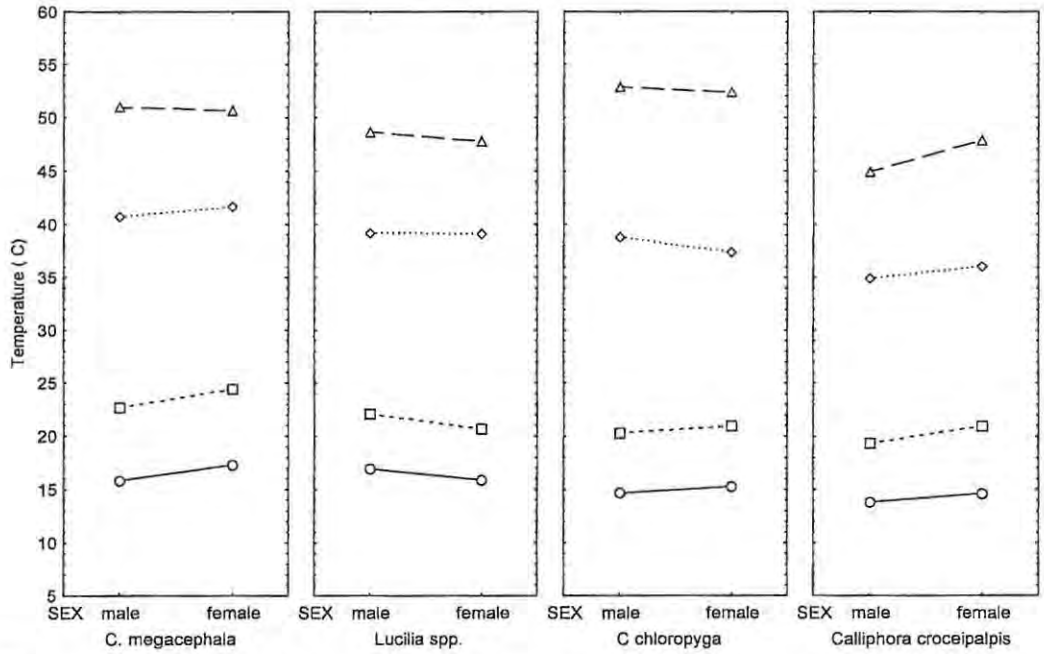

Figure 5.1: Mean critical temperature points in *Chrysomya megacephala*, *Lucilia* spp, *C. chloropyga* and *Calliphora croceipalpis* (temperature of movement = ○, temperature of walking = □, temperature of shade-seeking = ◊, temperature of death = Δ).

Table 5.1: Statistical values of ANOVA to show the size differences between male and female flies.

Species	N Males Females	F	P
Chrysomya megacephala	8 12	2.28	0.15
Lucilia spp	15 15	35.85	0.00
Chrysomya chloropyga	10 10	15.22	0.00
Calliphora croceipalpis	4 5	11.75	0.01

CHAPTER 6

Conclusion

Little work on blowflies in South Africa has been conducted in a forensic context (Prins, 1983). The work presented here will aid forensic entomologists in South Africa to perform their work more effectively.

Temperature and humidity are the major factors that control oviposition and rates of development of forensic insects and thereby their occurrence and activity (Smith, 1986). However, competition between some species may have an effect their occurrence. This is one of the reasons suggested as to why *Lucilia sericata* is less prominent in carcasses in summer than in winter (Tantawi et al., 1996). *Chrysomya albiceps* occurs in summer and reduces the numbers of *L. sericata* by direct competition for resources and by predation (Tantawi et al., 1996).

There are thermal gradients in which blowflies are active and above or below these ranges they seek shelter. Cold weather and rain inhibit fly activity (Smith, 1986, Chapters 2 & 5). Therefore in rainy and cold weather, flies will not be abundant as they are not active. This means that in the middle of summer a few cold or rainy days may reduce apparent blowfly occurrence. Thus, seasonal occurrence and nocturnal oviposition are affected by the temperatures at which blowflies are active.

As has been shown in previous succession studies, carrion exposed in summer attracts more species of insects than carcasses exposed in winter (Johnson, 1975; Cento, 2002). Clearly different insects occur at different times of year. Knowing what insects to expect is obviously important in forensic entomology to determine post mortem intervals (PMI). Seasonal occurrence of insects is therefore very important when bodies have been exposed for months.

Apparent seasonal occurrence of blowflies has been shown to vary according to the census method used (Chapter 2). Trap and carcass populations may differ by a few months depending on the species. *Lucilia* species and *Chrysomya chloropyga* were found in carcasses earlier than they were found in traps. *Chrysomya albiceps* and *C. marginalis* do not show the same trend. Obviously a corpse is not a trap, but a carcass,

and this phenomenon must therefore be taken into consideration when using seasonal data and must be determined for other species.

The zoogeographic region, country and local geographic zone within a country will affect the composition of the faunal succession on, and rate of decomposition of a carcass. In cooler polar and subarctic regions, fewer species of flies are found, whereas in more temperate regions the numbers increase greatly (Smith, 1986). It is important therefore to consider the geographic region and how this may affect the forensically important species distribution, particularly in succession studies (Smith, 1986).

Modelling of geographic distribution of blowflies to predict suitable areas of habitat is a potentially useful tool for forensic entomologists. By using records of known localities, the potential distribution of a species in intervening locations can be determined. The results of this work (Chapter 3) show that forensic entomology based on flies should be possible in principle almost anywhere in South Africa. The most important species however, may vary from place to place and season to season.

The geographic and seasonal distributions in some species are linked. *Calliphora croceipalpis* for example is found during winter and early spring (Fig 2.10) and it is found in the colder parts of the country (Fig 3.8). *Chrysomya putoria* occurs in summer when it is very hot (Fig 2.8) and is found in the tropical regions of South Africa (Fig 3.8) and further north in Africa. Thus certain predictions as to a species' geographic distribution can be made on the basis of its seasonal distribution, although this may not always be true or particularly precise.

The temperature ranges within which blowflies are able to survive are not necessarily linked to their seasonal or geographic distribution. *Chrysomya megacephala* is found in winter (Fig 2.9) yet it has a very high death threshold (Fig 5.1). However, *Calliphora croceipalpis* is also found in winter (Fig 2.10) and this species has a much lower death threshold (Fig 5.1). One must therefore be careful of assuming that flies have a low temperature threshold because of seasonality or that they must occur during summer because they have high thresholds.

Nocturnal oviposition has been examined in the recent years. In this work it has been shown to occur in certain flies in South Africa. Thus when determining PMI's, the possibility of nocturnal oviposition needs to be considered particularly when the weather is warm enough for blowflies to be active at night at anytime. This may limit the precision of PMI estimates, but the thermal limits can be used to adjust these.

Although temperature is clearly a major determinant of the temporal and spatial occurrence of blowflies, this relationship can be substantially modified by other ecological factors. The availability of particular feeding and breeding resources (Chapter 2) is a germane example.

There is still a great deal of work that needs to be done in South Africa for forensic entomology to become more commonly used. This work will hopefully serve as a foundation for future studies.

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