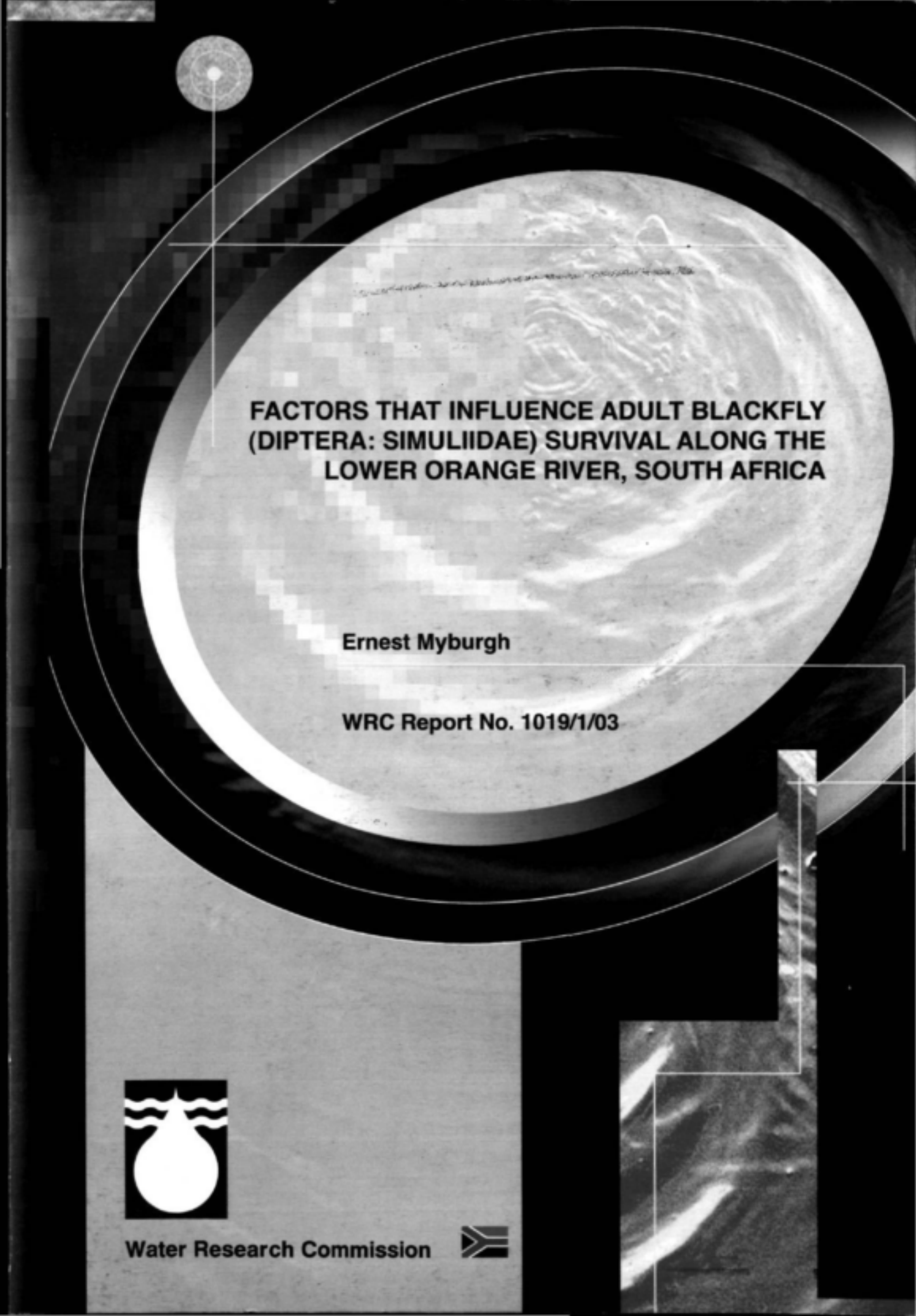

FACTORS THAT INFLUENCE ADULT BLACKFLY (DIPTERA: SIMULIIDAE) SURVIVAL ALONG THE LOWER ORANGE RIVER, SOUTH AFRICA

Ernest Myburgh

WRC Report No. 1019/1/03

Water Research Commission

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By

Ernest Myburgh

ARC-Onderstepoort Veterinary Institute (ARC-OVI)
Blackfly Field Station
Private Bag X5912, Upington, 8800, RSA

2002

Final report to the Water Research Commission on project K5/1019//4 entitled: "*The Orange River blackfly* Simulium chutteri: *Investigations into the physiology of the aquatic and non-aquatic stages so as to adjust the existing control programme to be more effective*"

Director of ARC-OVI: Dr Steven Corneluis
Project Leader: Dr Errol Nevill

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EXECUTIVE SUMMARY

Introduction

In 1996 the Northern Cape Agricultural Union estimated that blackflies accounted for up to R88 million per annum in lost sheep production along 800 km of the Orange River, and a further R19 million in lost taxes. The main pest is *Simulium chutteri* Lewis, a species endemic to Southern Africa. This species disperses up to 80 km on either side of the Orange River, depending on wind direction, and is a problem from approximately Hopetown to the mouth of the Orange River, including the Richtersveld and Southern Namibia.

Blackflies breed in rivers in a constant flow of fast-moving water where they attach to rocks and plants and filter out suspended particles. In the late 1970's the State built two large dams in the Orange River, namely the Gariep and Vanderkloof Dams. These made a great contribution towards the establishment and maintenance of irrigated crops throughout large sections of the river. The dams have, however, also resulted in a constant blackfly problem. Early attempts to control blackfly breeding relied on stopping the water flow from Vanderkloof Dam. However, the increasing need for irrigation water brought this control approach into conflict with farming requirements, so an alternative control approach had to be found.

In 1991 the ARC-OVI started large-scale larvicide trials, which led to the registration of two environmentally-safe products which are effective against most larval stages. Since 1993 an annual control programme has been funded and executed by the National Department of Agriculture, with entomological assistance from ARC-OVI. Under ideal conditions, if timed correctly and if the correct larvicide and dosage rates are applied, excellent blackfly control is achieved. However, sometimes failures occur and blackfly outbreaks take place. Some of these outbreaks can be attributed to human error, but our lack of knowledge on many fundamental fitness traits of, specifically adult *S. chutteri*, makes accurate planning of control actions difficult and inaccurate. The aim of this project was to address some of these aspects by studying various physiological and ecological factors that affect the survival of *S. chutteri*.

Aims

The aims of the project as stated originally were:

a) To compare the size and physiological state of larvae throughout a full year and relate to water temperature (Chapter 3);

b) To compare the size and physiological state of newly emerged adults throughout a full year and relate to final stage larvae (Chapter 3);

c) To compare the size and physiological state of wild-caught adults at varying distances from the river throughout a year and relate to newly emerged adults (Not completed – see below);

d) To determine the longevity and physiological state of newly emerged adults fed 5% sucrose/glucose and maintained at different temperatures and relative humidities in the laboratory throughout a year and relate to wild-caught adults (Chapter 4);

e) To select various representative vegetational transects along which to record plant species, density, foliage and flowering throughout a full year (Chapter 5);

f) To note and record the plants on which adult blackfly are seen resting and feeding (Chapter 5);

g) To determine the effect of light intensity, humidity, temperature and wind speed on adult host-seeking and blood feeding (Chapter 6);

h) To make use of the knowledge gained to adjust the annual control programme to be more effective (Chapters 8 and 9);

A further aim of the project was to:

i) Define adult blackfly annoyance on sheep (Chapter 7).

Objectives not addressed

Due to the unfortunate departure of Dr Moira Bode from the ARC-OVI, the adult blackflies collected at various distances from the river have not been analyzed, and therefore objective c (see page v) has been excluded from this report.

Summary of results

Strong inverse relationships were found to exist between developmental temperature and body size and mass in all the groups studied. Furthermore, pupae from Prieska were significantly larger than pupae from Gifkloof at corresponding times. The site differences were, however, no longer significant when developmental temperature was included as a co-variate. This suggests that temperature is the proximal factor affecting the size of *S. chutteri* pupae.

Strong linear relationships were found between lipid content and body mass in both pupae and adults. Furthermore, lipid content and developmental temperature were found to be inversely related in both pupae and adults. However, no significant relationship was found between glycogen content and body mass in either pupae or adults. The relationship between glycogen content and developmental temperature was also not significant.

Analyses of the *S. chutteri* groups used in the longevity trials showed that the winter population had the largest mean size and mass and the highest lipid and glycogen content. The spring population had the second highest size, mass, lipid and glycogen values, and size and metabolic reserves declined to summer, and then increased slightly in the autumn population.

Large variance was found in the longevity of the adult blackflies within and between populations and over the entire temperature and humidity scale tested. For example, longevity varied between less than 10 hours at high temperatures and low humidities to more than 20 days at low temperatures and high humidities.

Longevity in all the populations decreased exponentially with an increase in temperature and a decrease in relative humidity (R.H.). Despite these similarities, substantial variance was found in the longevity between the various populations. For example, the winter population was more sensitive to high temperatures than the spring population. The spring population furthermore showed much longer survival over the rest of the conditions tested and survived considerably longer at lower temperatures than the other populations. The summer population appeared to be less sensitive to high temperatures than the spring population, but more sensitive than the winter population. It was found that much of the variation between populations was a consequence of differences in survival at the lower temperatures and higher humidities.

Surprisingly, it was found that despite their large body size and metabolic reserves, the winter population showed the lowest tolerance to desiccation, starvation and heat. Therefore, the winter population showed comparatively low survival under a variety of conditions. It is argued that the low stress tolerance of this population is the result of autogeny that diverts energy and other resources from stress resistance.

The results of the phenological study showed that the 29 most abundant plant species in the Augrabies Falls National Park had a growth peak in late summer (January to March). However, the percentage of plant species flowering throughout the year stayed remarkably

constant, although small peaks were observed from January to March and during May, October and December. A total of 95 other flowering species were recorded in the AFNP during the study period.

Blackfly activity was recorded on the flowers of *Pappea capensis*, *Acacia karroo*, *Tamarix usneoides*, *Acacia mellifera*, *Ziziphus mucronata*, *Schotia afra* and *Sisyndite spartea*. Activity was more common on the first five species and they are therefore likely to be the preferred nectar sources. At least one of these species was flowering at any given time of the year. Resting behaviour was recorded on the seven species mentioned above, and on *Adenolobus garipensis*, *Phragmites* spp., *Diospyros lycioides*, *Ficus cordata* and *Rhus pendulina*.

The results of the climatological study showed that *S. chutteri* is active between 15.3 – 41.0 °C, although the optimum temperature for activity lies between 24 – 32 °C. Similarly, activity was recorded when the relative humidity (R.H) was between 4.5 and 53.5 %, but activity was highest when the R.H. was between 35 and 50 %. Furthermore, it was shown that *S. chutteri* is only active when the wind speed is between 0 and 4.1 m/s. Lastly, activity was recorded between light intensities of 30 and 9600 lux, which should be considered their tolerance levels.

In this study five distinct responses were recorded for sheep under attack from blackflies, including ear twitches, tail twitches, head shaking, skin rippling and feet stamping. Each of these repsonses had a specific purpose and served to deter attacks on various parts of the body. The only significant correlation between a behavioural response and adult numbers were recorded for the number of ear twitches. It was also shown, that within a herd of sheep, individuals use different methods of protection. It is argued that the less effective methods may enhance the probability of disease transmission.

Discussion and conclusion

The results of this study can be used to explain, to some extent, the typical seasonal variation found in the annoyance levels exhibited by adult blackflies along the lower Orange River and can therefore be used to predict outbreaks more accurately. During winter adult annoyance usually remains low, despite pupal abundance often being high. In this study it was shown that the autogenous nature of the winter population has a negative impact on adult survival by diverting energy and other resources away from important fitness traits such as stress resistance, and therefore they show comparatively low survival. Therefore, the importance of the winter population lies, not in its high annoyance levels, but rather in its ability to successfully deposit vast numbers of eggs in the Orange River - which leads to potentially major population outbreaks during spring.

Along the Orange River the largest outbreaks usually occur during spring each year. This present study showed that many factors are responsible for this increase in biting annoyance. Firstly, the spring population exhibited the highest desiccation and starvation tolerance of all the populations tested. This high tolerance to stresses suggest that they are physiologically best equipped to survive under a range of environmental conditions.

Moreover, the spring population had the second largest body size of all the blood-feeding populations. It is therefore likely that their high annoyance levels, in comparison to the other populations, can be attributed to their ability to disperse relatively far, which will potentially bring them into contact with more hosts. Furthermore, if they do more readily engage in host-seeking behaviour and feed more successfully than the other populations, an increase in biting frequency can be expected.

In addition to the above-mentioned factors, larval numbers are generally very high during spring, which means that vast numbers of adults will emerge from the Orange River at that time. During spring, environmental conditions are not as harsh as those experienced over the summer months, and the present study showed that climatological conditions are most favourable for activity at that time. This, together with the fact that one of their favourite, abundant and widespread nectar sources, *Acacia mellifera* flowers at this time of the year, enhance their survival.

The summer population had lower desiccation and starvation resistance than the spring and autumn populations, although this is the population that is most likely to be exposed to such harsh conditions. Moreover, the longevity of the longevity of the spring and autumn populations were less than 24 h at temperatures exceeding 27 ^{0}C. This temperature is frequently exceeded during the summer months and therefore it appears that the longevity of the summer population will be severely limited in summer. As the summer population also emerged with low metabolic reserves they would need to take a sugar meal as soon as possible to ensure their survival. This, in turn, exposes them to the harsh climatological conditions and should result in rapid mortality.

Therefore, the probability of a summer outbreak seems highly unlikely. However, as summer outbreaks do occur from time to time, it is clear that some populations are capable of utilizing other mechanisms than those studied here to overcome the harsh conditions, or they are favoured by factors such as favourable climatological conditions or the presence of nectar sources that possibly enhance their survival.

Although population outbreaks frequently occur over the autumn period along the Orange River, the problem is seldom as severe as that experienced in the spring months. This can in part be attributed to the fact that the individuals that developed during autumn are slightly smaller and have less lipid and glycogen reserves than the spring population. Therefore, the arguments discussed under the spring population are also relevant here, although the smaller size and less reserves result in lower annoyance levels.

The results of the annoyance trials should be seen as the basis for future studies on the defensive behaviour of sheep and the feeding success of blackflies. It is believed that the study on the defensive behaviour can be used to indicate the level of adult annoyance and may therefore be used, for example, to test the efficacy of various repellants in future studies.

Recommendations

1. It is recommended that larvicide applications be considered during the summer months, but only after and when:

    - An objective adult monitoring programme has been put in place to quantify the nature of the summer outbreaks. This will also assist in predicting longevity under field conditions;
    - Larval numbers warrant control (i.e. exceeding 100 000m^2);
    - Periods of favourable environmental conditions arise (i.e. low temperature and high RH) that will favour adult survival;
    - Studies have been conducted on the mechanisms used by *S. chutteri* to overcome harsh environmental conditions.
    - The effect of high water flow on larval and pupal populations is understood.

2. It is recommended that one dedicated and qualified person be appointed to take responsibility for all monitoring aspects, as objective and accurate monitoring is crucial to the success of the control programme.

3. If the monitoring system can be improved a more flexible approach should be adapted where the results of the monitoring should be incorporated in the planning of control actions.

4. Every effort should be made to effectively control the winter larval population as this population can lead to major outbreaks during spring.

5. It is recommended that the blackfly control operators be made aware of the limitations of high temperatures and low humidities on adult blackfly survival and that control actions be adjusted accordingly, i.e. that control actions be suspended when the longevity plots indicate blackfly longevity will severely be affected.

Capacity building

Qualifications obtained

1. Mr E. Myburgh, ARC-OVI researcher, obtained his MSc degree in Entomology in 2002 at the University of Pretoria with a thesis entitled: **The influence of developmental temperature on the survival of adult *Simulium chutteri* (Diptera: Simuliidae).**

2. Mr A.J.Karools, ARC-OVI research assistant, completed a course in **MS Word** at the Academy of Learning (Upington)

Publications

Myburgh, E. 1999. Control of the blackfly, *Simulium chutteri*, along the Orange River, South Africa. Journal of the South African Veterinary Association. 70(1): 44.

Myburgh, E. 2000. Blackflies in South Africa – their control and the fear of resistance. Journal of the South African Veterinary Association. 74(1): 36.

Myburgh, E., Bezuidenhout, H. and Nevill, E.M. 2001. The role of flowering plants in the survival of blackflies (Diptera: Simuliidae) along the lower Orange River, South Africa. Koedoe. 44(2): 63 – 70.

Talks and poster presentations

Myburgh, E. 1999. Blackflies in South Africa – their control and the fear of resistance. Congress of the Parasitological Congress of Southern Africa, Augrabies Falls National Park, South Africa.

Myburgh, E., Nevill, E.M. 2000. *Three decades of blackfly control in South Africa.* Conference on Blackflies in the New Millennium. Brock University, St. Catherines, Ontario, Canada. 17 – 21 June.

Publications in preparation

Myburgh, E., Chown, S.L. and Nevill, E.M. The impact of developmental temperature on the longevity of adult *Simulium chutteri* (Diptera: Simuliidae).

Myburgh, E., Chown, S.L. and Nevill, E.M. Review of blackfly (Diptera: Simuliidae) control in South Africa with particular emphasis on the Orange River.

Myburgh, E., Chown, S.L. and Nevill, E.M. The seasonallity of adult *Simulium chutteri* (Diptera: Simuliidae) – ecological and physiological perspectives.

Myburgh, E. and Nevill, E.M. The impact of selected climatological factors on the activity of adult blackflies (Diptera: Simuliidae) along the lower Orange River, South Africa.

Suggested areas of future research

- Test the hypothesis that autogeny decreases the survival of the winter population;
- Test the size, mass and physiological variations over a larger area;
- Study the nectar availability to blackflies away from the river;
- Study the effect of cultivated plants on the survival of adult blackflies;
- Study the mechanisms used by *S. chutteri* to overcome harsh environmental conditions;
- Develop and implement an effective adult blackfly monitoring system.

Archiving of data

All data will be housed at the Blackfly Field Station of the ARC-OVI in Upington. These include raw data of experiments and weekly monitoring, an herbarium and video cassettes.

ACKNOWLEDGEMENTS

I wish to thank the Water Research Commission and the Agricultural Research Council for funding this project and allowing me the opportunity to use the data for an MSc thesis. All the members of the Steering Committee are thanked for their valuable input and direction given to this project. A special word of thanks goes to Mr Andre Karools of the ARC-OVI for his valuable help and assistance. I leaned heavily on him during this project and his willingness to support is really appreciated. I thank my supervisor, Dr Errol Nevill, for initiating this study, keeping me focused, and his valuable input and enthusiasm throughout. Prof Steven Chown and Dr Jaco Klok assisted with the planning and completion of many trials, as well as much of the statistical analyses. Their help is gratefully acknowledged. The South African National Parks, and in particular Dr Hugo Bezuidenhout, is thanked for permission to work in the Augrabies Falls National Park. Mr Louis Kotzè provided us with trial animals, a trial site and accommodation during the annoyance trials. He is wholeheartedly thanked for this. Mr Stanley Chamberlain of the Department of Water Affairs and Forestry (Upington) is thanked for the excellent facilities he provided us with. Lastly, I wish to thank all my co-workers at the ARC-OVI, and all the assistants that played a role in the successful completion of this project.

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Medical, veterinary and economic importance of blackflies

In addition to feeding on sugar, which is used as a fuel for flight (Hocking, 1953; Davies et al, 1962; Hunter, 1977; Sutcliffe, 1986), anautogenous adult female blackflies also require a blood meal (Welton et al, 1987; Palmer, 1997; Gibson and Torr, 1999) for ovarian development (Davies and Peterson, 1956; Peterson, 1959; Crosskey, 1990). Because of their blood-feeding activity blackflies are considered ideal disease transmitters (Crosskey, 1990) and are best known for transmitting the filarial nematode worm *Onchocerca volvulus* to humans (Nelson, 1991; Davies, 1994; Hougard et al, 1997; Gibson and Torr, 1999). The resulting disease known as onchocerciasis or "river blindness" has left more than 20 million people infected and millions more blind in West Africa and South America (Rodriquez-Perez et al, 1995; Samba, 1995; Hougard et al, 1997; Molyneux and Davies, 1997). The bites of some blackfly species can also cause allergic reactions in certain people, a condition known as "blackfly fever" or simuliotoxicosis (Crosskey, 1990; Palmer, 1997). This is characterized by swelling, itching, haemorrhage and oedema (De Villiers, 1987), and requires medical attention in severe cases (Mason and Schemanshuck, 1990).

In animals, blackflies have been implicated in the spread of leucocytozoonosis, (Anderson and Voskuil, 1963; Crosskey, 1993), bovine onchocerciasis, (Crosskey, 1990; Hadi and Takaoka, 1995), the cytoplasmic polyhedrosis virus, the iridescent virus, vesicular stomatitis (Bernardo and Cupp, 1986; Bridges et al, 1997; Maré, 1998), avian trypanosomes (Crosskey, 1993), *Myxomatosis* (Williams and Williams, 1966; Kettle, 1984) and *Dirofilaria* species (Simmons et al, 1989). It has also been shown that allergic reactions to blackfly bites, similar to that described in humans, can lead to the death of cattle (Mason and Schemanshuck, 1990). In South Africa, simuliids have been implicated in the spread of two pathogens to animals. These are a *Chlamydia* sp., that causes blindness in sheep and abortion in cattle (De Moor, 1982a), and the Rift Valley Fever virus, which led to a major Rift Valley Fever outbreak between Prieska and Groblershoop in 1975 (McIntosh et al, 1980).

In livestock, blackflies readily attack the exposed parts of the body, e.g. the eyes, ears and teats (Anderson and Voskuil, 1963) and the resulting wounds are prone to secondary infections, which sometimes lead to the death of animals (Palmer, 1997). In addition, blackflies cause considerable irritation (annoyance) to livestock (Anderson and Voskuil, 1963; Crosskey, 1990; Kok et al, 1994). In South Africa, it is known that sheep under attack from blackflies will bundle together with their heads stuck underneath each other. During these periods the sheep don't feed or mate, and this results in a loss in weight gain and a reduction in lambing percentages (Palmer, 1997). In southern New Zealand and Canada the irritation value alone of the pest is high enough to have it classified as the most significant insect pest in these areas (Gibson and Torr, 1999). According to Edman and Simmons (1985) the biting annoyance of haematophagous *Simulium* species can even be severe enough to warrant large-scale control operations. Blackfly annoyance furthermore leads to economic losses through reduced efficiency of agricultural and industrial workers, interference in recreation, and reduced real estate values (Mason and Shemanshuck, 1990).

Although there are numerous reports of blackfly epidemics in South Africa, only Steenkamp (1972) has made a detailed study on the economic importance of the pest. He reported physical destruction of the teats on some cattle and a meaningful reduction in milk production in animals affected by blackflies of up to 35 kg milk per week per cow (30 - 50 % reduction). In poultry, he also found a significant reduction in egg production of eight eggs per 10 hens per week (10 - 15 % reduction). Other reports confirm that cattle can loose udders, and sheep their ears, as a result of secondary infections that develop in blackfly wounds (De Moor, 1986). During a more recent, though smaller-scale survey along the Vaal River, farmers reported that blackflies killed lambs, caused losses of 60 % in total farm stock production and reduced milk production by as much as 55 litres per cow per week (O'Keeffe, 1985). The Northern Cape Agricultural Union estimated that blackflies can potentially cause losses estimated at more than R88 million per annum to the stock industry along the Orange River (Palmer, 1997). This figure is based on a 25% reduction in lamb production and excludes other figures such as land depreciation and tax losses to the State.

1.2 Current situation in South Africa

Prior to the building of dams, canals, irrigation schemes and hydro-electrical plants blackflies were not considered significant pests in South Africa. However, soon after the completion of such structures, blackfly problems arose (Nevill, 1988). This occurred partly because these impoundments promote eutrophication and the build-up of suspended organic material which create ideal conditions for immature blackflies (Howell and Holmes, 1969; Car, 1983; Nevill, 1988) to increase in numbers in rapids downstream of such structures (Chutter, 1963; Palmer, 1991). Today, blackflies are common pests along the Orange, Vaal, Great Fish, Sundays, Gamtoos and Eerste Rivers. Periodic outbreaks are also experienced along some of the smaller rivers, e.g. the Olifants and Berg Rivers (Edwardes and Palmer, 1994; Palmer, 1997) and it is expected that blackflies may acquire pest status along the Liebenbergsvlei River following the completion of the Lesotho Highlands Water Scheme (Fig. 1). Currently 39 blackfly species are known to occur in southern Africa (Palmer, 1997; Palmer and De Moor; 1998). These include five non-pest

Paracnephia species and 34 *Simulium* species (Palmer, 1997). The latter group includes the mammalian pests *S. chutteri* and *S. damnosum* s.l. and the avian pest species *S. adersi* and *S. nigritarse* s.l.

S. chutteri is considered to be the most important blackfly pest species in South Africa. It occurs along the Vaal, Great Fish and Sundays Rivers, but is most abundant and causes the largest economic problems along the lower Orange River (Palmer, 1997). *S. chutteri* is a large-river species endemic to southern Africa which, under favourable conditions, can become the most abundant blackfly species in this region with larval densities exceeding 500 000 m^2 (Palmer and De Moor, 1998). It is a multivoltine species with 11 to 13 generations per annum (Palmer et al, 1996). *S. chutteri* occurs throughout the year although an increase in biting activity is usually experienced in spring and early summer (August – November) and autumn (April - May) (Jordaan and Van Ark, 1990; Palmer et al, 1995b) suggesting that it is a species adapted to moderate weather conditions.

1.3 Blackfly control in South Africa

The first recorded blackfly outbreak in South Africa was in the vicinity of Wynberg in 1899 (Fuller, 1899, cited by Palmer, 1997) followed by a second outbreak in the area between Port St. Johns and Umtata (Fuller, 1913, cited by Palmer, 1997). According to Howell and Holmes (1969) periodic outbreaks were also experienced along the Vaal River prior to 1940. However, it was only after the completion of the Vaal Barrage (1923), Vaalhartz Diversion Weir (1936) and Vaal Dam (1938) (Fig. 1) that blackfly numbers steadily started to increase along the Vaal River (De Moor, 1986) and eventually developed pest proportions (Howell and Holmes, 1969).

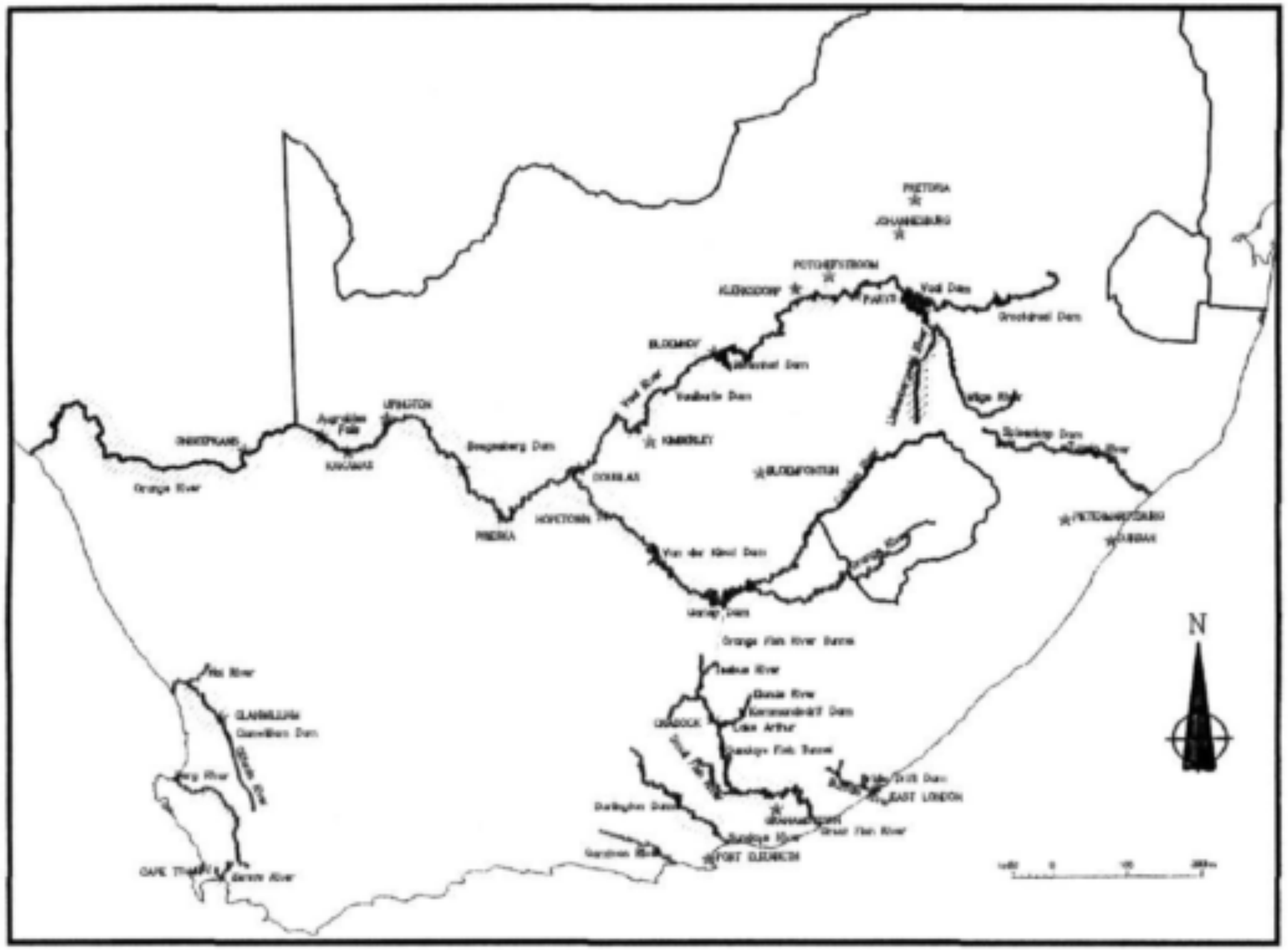


Fig. 1. Map of known blackfly problem areas in South Africa.

ince 1950 frequent blackfly outbreaks have been reported from the Vaal River (Howell and Holmes, 1969; Nevill, 1988) and subsequently *S. chutteri* (Chutter, 1968; Howell and Holmes, 1969) *S. damnosum* s.l., *S. nigritarse* (Steenkamp, 1972) and *S. adersi* (Begemann, 1980) were identified as pest species. After the 1963 *S. chutteri* outbreak in the Warrenton District (Chutter, 1968; Howell and Holmes, 1969) extensive studies were undertaken on the ecological requirements of simuliids (Chutter, 1968). These were followed in 1965 by the first attempts to control the pest by means of DDT. At that time DDT was considered "the perfect weapon for the perfect target" (Brown, 1962)!

Between 1965 and 1967 DDT was applied numerous times to the Vaal River from structures suspended above sluicegates or by fixed-wing aircraft (Howell and Holmes,1969). The DDT applications resulted in the growth of benthic algae on rocks, which Car and De Moor (1984) attributed to the eradication of most invertebrates. Nevill (1988) noted that the algal mats had the benefit that they did not allow blackfly larvae to reattach to affected rocks. Although high mortalities were obtained with DDT, rapid larval reinfestation was recorded following the disappearance of the algal mats (Howell and Holmes, 1969). Owing to the environmental damage caused by DDT the control programme was suspended in 1967. However, after major floods in 1974, Begemann (1980), again found blackflies in great numbers in the Vaal River.

The 1970's saw the spread of the blackfly problem along the Vaal River after the completion of the Bloemhof Dam in 1970 (Car, 1983). During the period 1972 to 1978 the Gariep and Van der Kloof Dams were completed in the Orange River. This allowed *S. chutteri* to also develop to pest proportions along the lower Orange River (Nevill, 1988; Jordaan and Van Ark, 1990). In 1975 the Orange Fish Tunnel, linking the Gariep Dam to the Great Fish River (Fig. 1), was completed and reports indicated that *S. chutteri* also developed pest status in the Great Fish River during the years following the completion of the tunnel (Coetzee, 1982; O'Keeffe, 1985).

These problems led to the second phase in the battle against blackflies, namely the use of water flow manipulation. Water flow manipulation is the process by which the water levels of rivers are artificially fluctuated to expose and desiccate the sessile blackfly pupae as well as forcing the larvae to move to undesirable sites where they are prone to starvation and predation (Howell et al, 1981). Howell et al (1981) started trials in 1977 at the Vaalhartz Diversion Weir and found a drop in the numbers of immature blackflies for up to 30 km downstream of the weir. They followed this with trials in the Orange River during 1978 at the Van der Kloof Dam where water flow was interrupted for approximately 66 hours. Here they reported similar successes. The authors recommended that cut-offs in water flow be implemented twice annually, during May and August. They furthermore claimed that *S. chutteri* lost its pest status in sections of the river where regular water flow fluctuations were implemented. Trials by Car (1983) confirmed that a reduction in the water level of the Orange River reduced the number of immature blackflies in the river. The greatest effect on larvae could be found during winter and he recommended a cut-off in water flow during July/August when the majority of the population is in the larval phase.

During the same period De Moor (1982a; b), working along the Vaal River, proposed a third method of blackfly control. This method involved an integrated approach where data on the life-cycle, population dynamics and microhabitat preferences of the six most abundant *Simulium* species, and their natural aquatic invertebrate predators, were used to determine the best time to carry out a series of river-flow cessations. Water flow regulation was then applied to halt the build-up of populations and maintain *S. chutteri* at levels at which they could be controlled by natural predators. Although integrated water flow manipulation can be regarded as the most cost-efficient and ecologically least disruptive of the available methods, De Moor and Car (1986) argued that the method is limited by the availability of impoundments upstream of *Simulium* breeding sites.

At this time there were major agricultural developments along the Orange River with the expansion of the traditional crops to include winter crops such as wheat and peas. Nevill (1988) concluded that the additional irrigation requirements and hydro-electricity demands made the further use of water flow manipulation impractical along the Orange River. Researchers also realized that the long distances over which water flow had to be manipulated made the sustainable use of this method impractical (Jordaan and Van Ark, 1990).

During the 1980's *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (serotype H-14) (*Bti*) was gaining ground as a biological agent for the control of simuliids after studies by Undeen et al (1981) and Lacey et al (1982) indicated that this method of control was effective. Following these reports, Car and De Moor (1984) conducted trials during 1982 in the Vaal River and reported high larval blackfly mortalities. Laboratory and field trials by Car (1984) also showed that *Bti* is effective in controlling blackflies, but that its toxicity was considerably reduced in polluted rivers with a high sewage level and high chloride concentration. Subsequent trials in the Orange River during 1983 confirmed the efficacy of *Bti* against blackflies and its low toxicity to non-target organisms (De Moor and Car, 1986).

Large-scale river trials were started during 1991 with an improved, more practical *Bti* formulation and the organophosphate temephos. Both proved to be effective (Palmer, 1995). Various studies on the impact of these two larvicides on non-target organisms showed that they were safe for use along the Orange River (Palmer, 1993; Palmer and Palmer, 1995). At the same time trials were done to assess the downstream carry of these two larvicides (Palmer et al, 1995a) and on the timing of larvicide applications (Palmer et al, 1995b). Furthermore, methods were developed for rapidly assessing larval and pupal abundance before and after larvicide applications (Palmer, 1994).

During 1992 the Orange River Blackfly Control Programme (ORBCP) was launched. Currently the ORBCP is implemented by the National Department of Agriculture. Between 2 to 19 temephos and *Bti* applications are needed annually to control the pest (Palmer, 1997; Myburgh unpublished data 2000). For detailed reviews of the ORBCP see Palmer et al (1996), Palmer (1997) and Palmer (1998).

1.4 Purpose of the present study

Although a blackfly control programme is in place along the lower Orange River, major outbreaks still occur (Palmer, 1997; Myburgh, 1999). Some of these outbreaks can be attributed to human error (e.g. unavailability of helicopters, late ordering of larvicides, etc), but a lack of information on several population dynamic factors makes planning of control actions difficult and inaccurate.

In the current planning of control actions, fundamental fitness traits such as fecundity, dispersal, feeding, survival and longevity are ignored, although studies elsewhere have shown that these are all important considerations in the planning of control actions (De Moor, 1982b; Colbo and Porter, 1979). Of these factors, longevity is probably the most important as it has a bearing on how many times a female can take a blood meal and thus indirectly influences her biting rate and the damage caused to host species. Longevity is, however, a complex variable because an adult blackfly that emerges from the river is subject to prevailing climatic conditions. The effect of these climatic conditions, which will alter with the seasons, will be modified by the physiological state of the fly (Colbo, 1982; Ross and Merrit, 1987), which in turn depends on the water temperature at which the larvae develop (Colbo, 1982; Van Handel, 1985a; Nasci, 1991; Roff, 1992; Stearns, 1992; Thomas, 1993; Hancock and Foster, 1997).

Therefore, to be able to more accurately plan control actions it is necessary to: (a) be able to predict adult longevity under a variety of environmental conditions, and (b) understand how adult longevity is influenced by the water temperature experienced by larvae. To accomplish this goal the following hypothesis was tested: Since developmental (i.e. water) temperature is a key determinant of ectotherm size in nature (Atkinson, 1994; Chown and Gaston, 1999), the size of *S. chutteri* should vary with seasonal and geographical changes in water temperature. These changes in body size should be reflected in variable nutrient reserves contained within adult flies, and these in turn should have an affect on longevity (Service et al, 1985; Graves et al, 1992). Larger flies, with more reserves, should show increased survival and be able to withstand various environmental conditions more effectively than smaller specimens with less reserves. In consequence, flies emerging at a time of the year, following development at a particular temperature, favourable for the production of larger flies with greater metabolic reserves, should have a considerably greater longevity than those emerging following development when conditions were less favourable.

Furthermore, blackflies need carbohydrates for survival, but it is thought that the arid conditions along the Orange River may severely limit nectar availability during certain periods (Palmer 1997). To test this the nectar availability in the Augrabies Falls National Park was studied over a full year and related to adult survival.

Lastly the relationship of various climatological factors to adult feeding activity were studied, as this could determine the actual level of annoyance of a given number of blackflies.

1.4.1 Original objectives as stated in research proposal

- To compare the size and physiological state of larvae throughout a full year and relate to water temperature (See Chapter 3);
- To compare the size and physiological state of newly emerged adults throughout a full year and relate to final instar larvae (See Chapter 3);
- To determine the longevity and physiological state, throughout a full year, of newly emerged adults fed 5 % sucrose/glucose and maintained at different temperatures and relative humidities in the laboratory and relate these findings to wild-caught adults (See Chapter 4);
- To compare the size and physiological state of wild-caught adults at varying distances from the river throughout a full year and relate to newly emerged adults (Not completed – see 1.4.3);
- To note and record the plants on which adult blackfly are seen resting and feeding;
- To select various representative vegetational transects along which to record plant species, density, foliage and flowering throughout a year (See Chapter 5);
- To determine the effect of light intensity, humidity, temperature and wind speed on adults host-seeking and blood-feeding (See Chapter 6);
- To make use of the knowledge gained to adjust the annual control programme to be more effective (See Chapters 8 and 9).

1.4.2 Additional objectives addressed

- To define the annoyance levels of blackflies on sheep (See Chapter 7).

1.4.3 Objectives not addressed

Due to the unfortunate departure of Dr Moira Bode from the ARC-OVI, who was responsible for the lipid and glycogen analyses, the adult blackflies collected at various distances from the river have not been analyzed, and therefore objective c (see page v) has been excluded from this report.

2. BROAD DESCRIPTION OF STUDY AREA

This study was conducted at the Agricultural Research Council - Onderstepoort Veterinary Institute's (ARC-OVI) Blackfly Field Station (28° 28' 14"S, 21° 15' 37"E) at the Department of Water Affairs and Forestry in Upington (Northern Cape Province, South Africa) (Fig. 2.1). The Blackfly Field Station was established in 1991 to facilitate studies on Orange River blackflies.

Gifkloof (28°26'00"S; 21°23'21"E), located approximately 20 km east of the Blackfly Field Station, was used as the primary study site. The site consists of a series of small rapids that has been utilized as a control and research site for more than 10 years by blackfly researchers. Gifkloof is excluded from all large-scale larvicide applications as it acts as a control area and refuge for non-target organisms in the event of accidental overdosing. Prieska (29°39'38.70"S; 22°45'20.67"E), located 260 km upstream from Gifkloof, was chosen as the secondary study site. The Prieska site comprises a single set of rapids that are treated during blackfly control operations. Water temperature at Prieska is colder on average than at Gifkloof (Palmer, 1997) (Fig. 2.1).

It is known that the Orange River supports large populations of *S. chutteri*, from the Gariep Dam to Vioolsdrift, a total distance of 1470 km (Palmer, 1997). There is a strong water temperature gradient along the river (Chutter et al, 1996) and annual means vary between 18.2 °C at Bleskop to 22.5 °C at Vioolsdrift (Everson, 1999) (Fig. 2.1). Major seasonal shifts occur in water temperature along the Orange River with summer highs of 30 – 35 °C and winter lows of 5 – 15 °C (Everson, 1999).

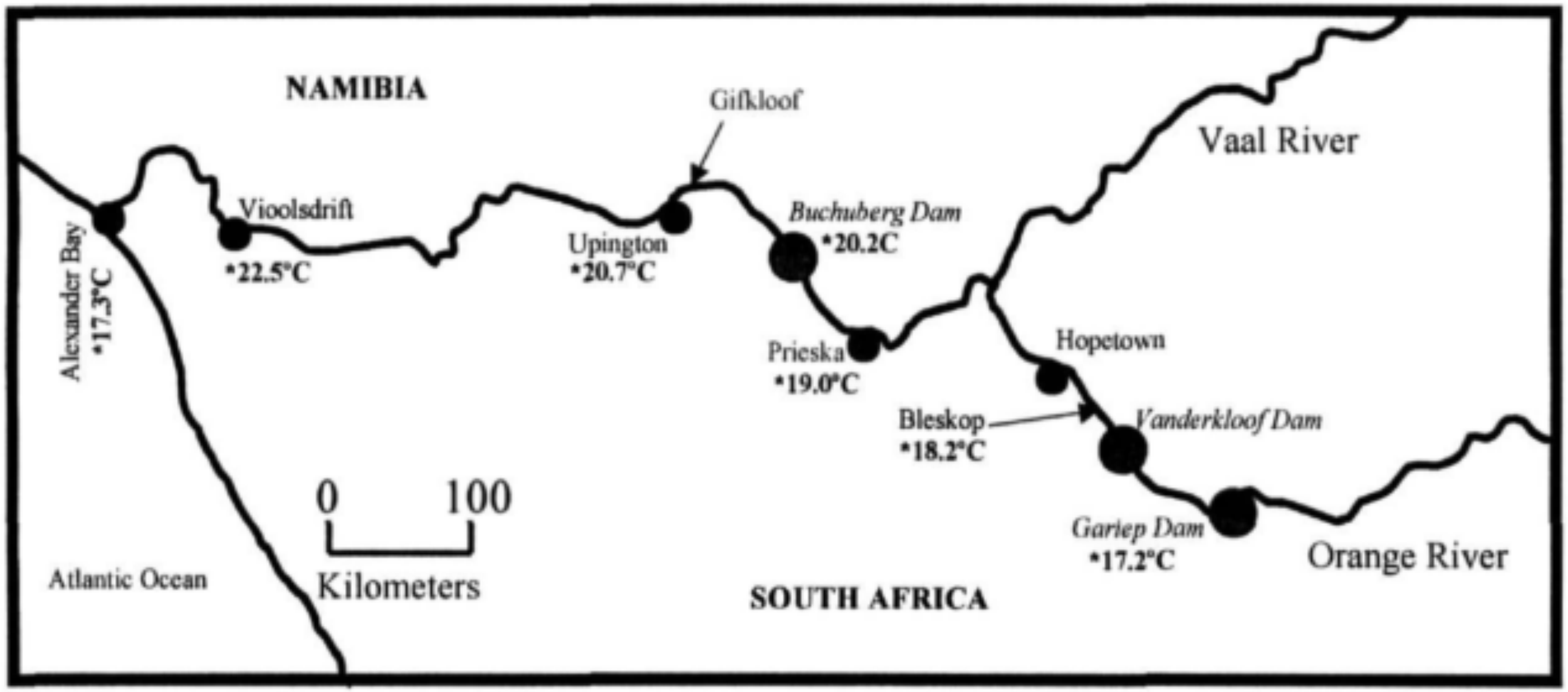


Fig. 2.1. Map of the study area showing the position of the study sites, impoundments and mean annual water temperature at various sites (Map redrawn from Palmer, 1997. Temperature data from Chutter et al, 1996; Everson, 1999).

Average weekly temperatures recorded at Gifkloof from January 1991 – June 1999 show summer highs of 26 – 28 °C and winter lows between 9 – 11 °C (Palmer, 1997; Myburgh, unpublished data, 2000) (Fig. 2.2). The Orange River has a mean discharge of 100 m^3 and is mostly between 100 – 300 m wide, although it can be as narrow as 10 m in certain stretches and more than 3 km wide in severely braided sections (Palmer, 1997). The Orange River supports irrigation farming, and grapes, lucerne and cotton are commonly grown. Domestic stock in the area include sheep, cattle, goats, horses, donkeys and ostriches (Department of Agriculture, Upington).

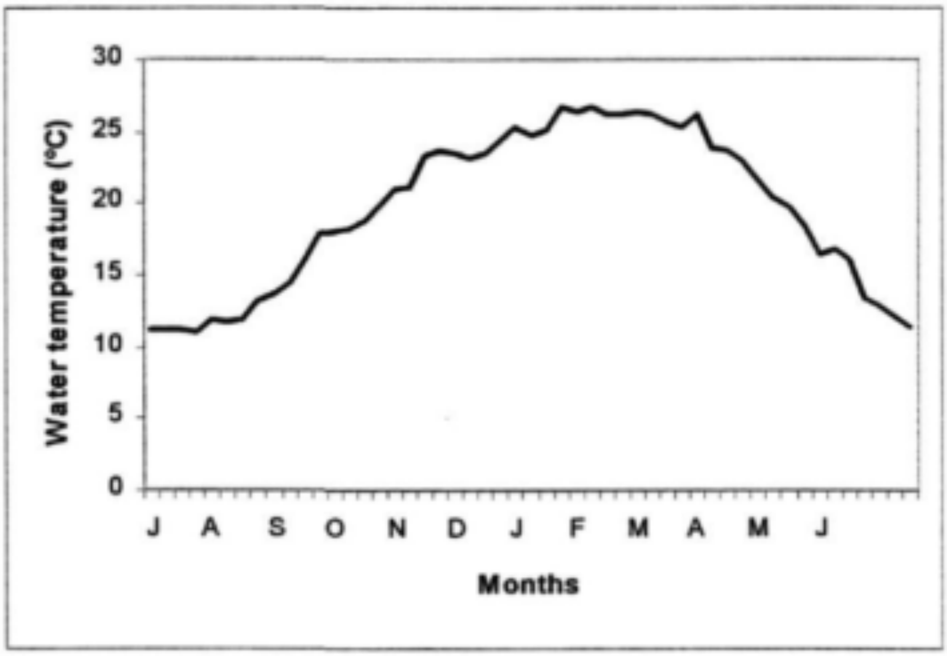


Fig. 2.2. Weekly average water temperature at Gifkloof for the period January 1991 to May 1999 (Palmer, 1997; Myburgh, unpublished data, 2000).

At Upington air temperature varies between 43 °C in summer and – 5 °C in winter. Seasonal changes in minimum and maximum temperatures at selected sites are given in Table 2. The relative humidity at Upington is low with an annual mean of 28 – 30 %. The study area falls in the summer rainfall area with an annual average of 150 mm. Prevailing winds are from the north in winter (May – August) and from the south-west in summer (September – March) (Department of Environmental Affairs and Tourism, Upington).

Table 2. Seasonal changes in average daily minimum and maximum air temperatures (°C) at selected sites (Department of Environmental Affairs and Forestry).

Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Douglas	17.8	17.6	15.0	10.7	5.4	1.6	1.0	3.4	7.6	11.2	14.1	16.4
	34.9	32.9	30.6	26.4	23.3	19.7	20.2	22.4	25.7	28.7	31.6	33.6
Prieska	18.9	18.1	15.6	10.8	5.4	1.5	1.1	3.3	7.7	11.3	14.9	17.5
	35.0	33.6	31.2	26.7	22.7	19.1	19.6	21.9	26.2	28.8	31.8	34.0
Upington	19.8	19.5	17.8	13.3	8.3	4.8	4.1	5.6	9.4	12.9	16.3	18.6
	35.5	34.4	32.1	27.8	24.0	20.5	20.8	22.9	26.8	26.8	32.7	34.7
Kakamas	21.8	21.6	20.2	16.4	11.2	7.1	6.4	8.7	12.0	15.6	17.9	20.6
	35.7	34.6	32.3	29.7	24.4	21.8	20.9	23.7	26.6	30.5	33.0	35.6

The phenological study was conducted in the southern section (4 500 ha) of the Augrabies Falls National Park (AFNP). AFNP is located approximately 120km west of Upington (28°25'-28°38'S; 20°15'-20°20'E) (Fig. 2.1). The AFNP has a history of blackfly attacks, and the Augrabies gorge is considered one of the main breeding sites for blackfly along the Orange River (Palmer 1997).

The air temperature in the AFNP varies between –2.9°C in winter and 42.9°C in summer (Land Type Survey Staff 1986). The park falls in the summer rainfall area and averages 211mm per annum, but it has been shown to vary between less than 40mm to 391mm per annum (Weather Bureau 1996). Rainfall and temperature figures during this study were obtained from the Weather Bureau's data collected in the AFNP.

The vegetation in the park is typical of the Orange River Nama Karoo Type found within the drainage basin of the Orange River. This vegetation is found in a band (approximately 10 – 75km wide) along the Orange River and extends from Vioolsdrift in the west to Hopetown in the east (Hoffman 1996). The AFNP is the largest conservation area within the Orange River Nama Karoo biome and contains six major plant communities, namely *Aloe dichotoma* Sparse Woodland, *Schotia afra* Open Woodland, *Ceraria namaquensis* Open Shrubland, *Acacia mellifera* Open Shrubland, *Stipagrostis hochstetteriana* Open Grassland and *Ziziphus mucronata* Closed Woodland (Bezuidenhout 1996).

3. INFLUENCE OF DEVELOPMENTAL TEMPERATURE ON THE SIZE, MASS AND METABOLIC RESERVES OF *S. CHUTTERI*

3.1 Introduction

Norms of reaction that map adult ectotherm size on developmental temperature are remarkably consistent in shape, and these two variables are generally inversely related (Atkinson, 1994; Berrigan and Charnov, 1994; Van der Have and De Jong, 1996; Atkinson and Sibly, 1997; French et al, 1998). Final body size of most ectotherms therefore increases with a decrease in developmental temperature, although some studies have shown that the relationship can become curvilinear below a certain thermal threshold (Kari and Huey, 2000). Developmental temperature is also integrally linked to developmental time (Bates, 1947; Van der Have and De Jong, 1996) and ectotherms developing at colder temperatures generally show decreased development and reach maturity later at a larger size than specimens developing at warmer temperatures (Berrigan and Charnov, 1994; French et al, 1998). Whether or not these norms of reactions are adaptive responses or contingent processes (non-adaptive responses) is still heavily debated and researchers seem far from general agreement (Scheiner, 1993; Via, 1993; Atkinson, 1994; Atkinson and Sibly, 1997). Nonetheless, the generality of the relationship between body size and developmental temperature is so strong that Atkinson (1994) suggested that it is, in fact, a "biological law".

The relationship between developmental temperature and metabolic reserves is also inverse in a wide range of ectotherms (Van Handel and Day, 1988; Briegel, 1990; Naksathit and Scott, 1998; Takken et al, 1998). More specifically, specimens that develop at colder temperatures generally carry more lipid, protein and carbohydrate reserves than specimens developing at warmer temperatures (Takken et al, 1998; Briegel, 1990). In addition, several previous studies have demonstrated that lipid and glycogen content are positively correlated with the longevity of ectotherms (Service et al, 1985; 1988; Service, 1987; Graves et al, 1992; Burgin and Hunter, 1997a; Sawabe and Mogi, 1999). Therefore, it can be concluded that larger specimens generally carry more lipid and glycogen reserves and therefore survive longer than smaller specimens (Bates, 1947; Joshi, 1995; Takken et al, 1998). Thus, it is clear that in many small ectotherms longevity is strongly influenced by metabolic energy reserves, which are, in turn, closely linked to body size (Bates, 1947; Joshi, 1995; Takken et al, 1998), and that both body size and the amount of metabolic reserves are dependent on developmental temperature (Bursell, 1974; Beck, 1983; Joshi, 1995).

Because there are considerable seasonal and geographical changes in the water temperature of the Orange River (see Chapter 2), adult body size and metabolic reserves of *Simulium chutteri* should vary with season and locality. Thus, the first step to understanding seasonal variation in the longevity of this species is to comprehend the extent to which seasonal variation in development temperature of the larvae might be responsible for variation in adult body size, mass and metabolic reserves. To determine this, variation in body size, mass and lipid and glycogen contents of pupal and adult *S. chutteri* were examined at the Gifkloof site. As an additional test of the influence of water temperature on body size, comparisons between two geographically distinct populations (Gifkloof and Prieska), which develop at different temperatures (see Chapter 2), were also made.

3.2 Materials and methods

3.2.1 Collection and rearing of specimens

Pupae were collected at 28-day intervals from July 1999 to August 2000. Unfortunately, in some months pupae were unavailable owing to severe flooding or algal blooms, which respectively, made the river inaccessible or induced high blackfly mortality (E. Myburgh, pers. obs.). In addition, pupae were absent during some periods at the Prieska site as a result of the blackfly control programme. Various attempts to hatch adults from Prieska pupae at the Blackfly Field Station were unsuccessful and this resulted in the exclusion of a geographic comparison of the adult flies.

Samples were obtained by collecting rocks from the two study sites and transporting them to the Blackfly Field Station. Here all blackfly larvae and other organisms were washed from the rocks to leave only blackfly pupae. For pupal studies, pupae were picked from the rocks, removed from their pupal cases, sexed and identified using the identification keys of Palmer (1991). Adults were obtained by placing washed rocks in emergence chambers. These chambers were developed for the purpose of this study and consisted of 15 L plastic containers with a funnel attached to one side. Rocks were placed inside the container and the chamber was then sealed and held in a shaded area under ambient temperature and light conditions. Adults that emerged were collected in styrofoam cups at the end of the funnel. Cups were removed every hour and rocks were replaced every 48 hours. Adults were subsequently identified and sexed.

3.2.2. Calculation of developmental temperature

Weekly water temperatures from Prieska were obtained from the Department of Water Affairs and Forestry (Upington), while water temperatures at Gifkloof were recorded using a hand-held mercury thermometer, during weekly sampling by ARC-OVI staff. Developmental time (egg to pupae) of *S. chutteri* varies with changing water temperature (Palmer, 1997), and therefore the developmental period for each sample had to be calculated using Table 3.1. The developmental temperature for each sample was determined by calculating the average weekly water temperature over the developmental period of each population. It should be noted that these are only approximate indices of actual developmental temperature, because they do not include diurnal changes in water temperature.

3.2.3 Size and mass analyses

For size measurements, 50 specimens from each sample were used. Pupal size was recorded as the length from the respiratory histoblast to the tip of the wing along a straight line (De Moor, 1982b) (Fig. 3.1.1) under a Wild M5 dissection microscope with a Wild 10X/21 calibrated micrometer eyepiece. Wing length is considered the best measure of adult size in simuliids (Crosskey, 1990), and in this study it was recorded as the length of the costal vein between the humeral crossvein and the point where the subcostal joins the costal vein (Fig. 3.1.2) (De Moor, 1982b). All size measurements were made to the nearest 1 µm. For mass measurements, ten groups of 10 specimens, from each sample were weighed on an Adam Equipment ESJ - 180 micro-balance (accurate to 0.0001 g).

Unless otherwise stated, the sample unit for the statistical analyses involving size measurements is the individual specimen (i.e. $n = 50$), while for the mass measurements the sample unit was the group of 10 individuals (i.e. $n = 10$). Least squares linear regression analyses were used to investigate the relationship between developmental temperature and size, and developmental temperature and body mass of adults and pupae at the Gifkloof site. To examine site-related (geographic) size differences, pupae from Gifkloof and Prieska were compared using an analysis of variance (ANOVA). In a subsequent analysis, water temperature was included as a covariate to determine its effect on body size. These analyses were done with STATISTICA (Statistica Statsoft, 1991).

Table 3.1. Approximate developmental period (in weeks) of *S. chutteri* larvae at various times of the year (After Palmer, 1997).

	Week			
Month	**1**	**2**	**3**	**4**
January	2	2	2	2
February	2	2	2	2
March	2	2	2	2
April	2	2	2	2
May	2	3	3	3
June	3	4	4	4
July	4	4	4	4
August	4	4	3	3
September	3	3	2	2
October	2	2	2	2
November	2	2	2	2
December	2	2	2	2

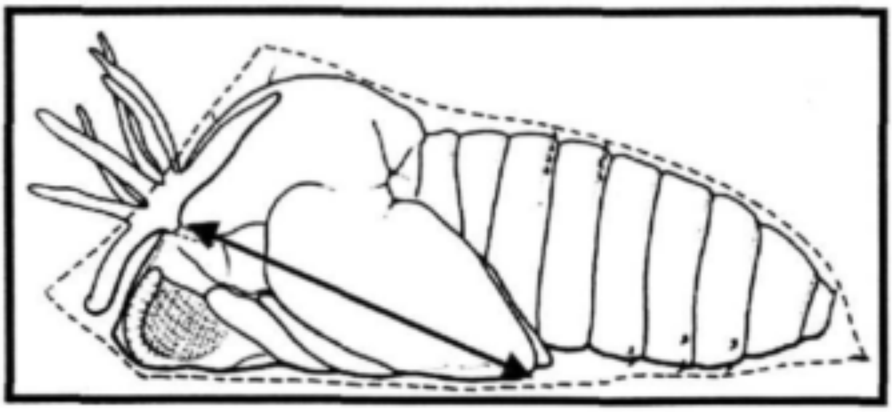


Fig. 3.1.1. Landmarks for measurement of the size of pupal *S. chutteri.*

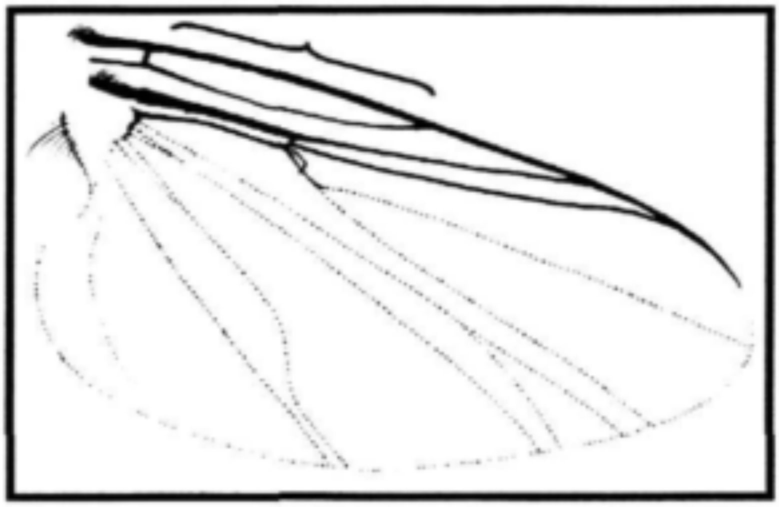


Fig. 3.1.2. Landmarks for measurement of the size of adult *S. chutteri*.

3.2.4. Lipid and glycogen analyses

A sample size of five groups of four individuals each was used for all lipid and glycogen analyses. For lipid analyses the method of Van Handel (1985a) was used. For each sample four blackflies were grouped, weighed on a micro-balance (Shimadzu Libror AE X-200B, std deviation ≤ 0,1 mg) and placed in 16 × 100 mm culture tubes. The blackflies were crushed with a glass rod in 0.5 ml chloroform-methanol (1:1). After the tubes were gently shaken the supernatant was transferred to clean tubes. These tubes were placed in an aluminum heating block at 90 °C to evaporate the solvent. Then 0.2 ml 95 % sulphuric acid was added and heated for 10 min. After cooling the tubes were filled to 5 ml with vanillin reagent, shaken and allowed at least 5 min for colour development before the tubes were read directly in a spectrophotometer (Shimadzu UV-260, Drift 0,0004 Abs/hr or less) at 525 nm against a reagent blank. When necessary, dilutions were made using reagent blank. Vanillin reagent was prepared by dissolving 600 mg vanillin in 100 ml hot water and then adding 400 ml 95 % sulphuric acid.

The total lipid content per sample (µg lipid/mg sample mass) was read directly from the calibration line, which was obtained by pipetting 50, 75, 100, 150 and 175 µl standard solution (100 mg per 100 ml commercial soy bean oil in chloroform) in tubes and evaporating the solvent. Thereafter it was treated as described above.

For glycogen analyses the method of Van Handel (1965b) was used. For calibration 25, 50, 75, 100 and 125 µg glucose solutions (1 mg/ml water in 25 % ethanol) were pipetted into culture tubes and 5 ml anthrone reagent was added, mixed, and heated for 17 min in a tube heater (90 – 92 °C). After cooling, optical densities were read on the spectrophotometer at 625 nm and calibration lines were plotted for µg glucose vs optical density. Anthrone reagent was prepared by pouring 150 ml water into a 1 litre Erlenmeyer flask. While cooling 380 ml concentrated sulphuric acid was added. Then 750 mg anthrone was dissolved in the diluted sulphuric acid.

For the determination of glycogen content in each sample, four blackflies were grouped, weighed, placed in culture tubes and 0.2 ml sodium sulfate (2 % solution in water) was added. The blackflies were then crushed with a glass rod. Then 1 ml methanol was added, vortex mixed and centrifuged for 1 min after which the supernatant was removed. 5 ml anthrone reagent was added to each tube, mixed, heated for 17 min, cooled and mixed again. These solutions were then read on the spectrophotometer at 625 nm. When necessary, dilutions were made using reagent blank. Total glycogen content per sample (µg glycogen/mg sample mass) was read from the glucose calibration line.

The lipid and glycogen (mg/mg body mass) data were converted to percentage lipid and glycogen per sample. Least squares linear regressions were used to investigate the relationship between the percentage lipids per sample and average body mass and the percentage glycogen content per sample and average body mass. Body mass used in these analyses is the average mass obtained from corresponding samples as calculated in Section 3.2.3. Least squares linear regressions were also used to investigate the relationship between the percentage glycogen per sample and developmental temperature and the percentage lipid per sample and developmental temperature.

3.3 Results

3.3.1 Water temperature

Water temperature showed major seasonal fluctuations at both sites (Fig. 3.2). Water temperature was lowest from June to August (mid-winter) at both sites and highest from November to March (mid-summer) at Gifkloof and highest from December to March at Prieska. Water temperature at Prieska was on average 2 – 3 °C lower than the temperature at Gifkloof throughout the year. The lowest recorded temperature at Gifkloof was 11 °C and the highest 28 °C. The corresponding figures for Prieska were 9 °C and 27 °C.

3.3.2 Size and mass measurements

Both body size and mass of pupae and adults from Prieska and Gifkloof varied over the study period (Figs. 3.3.1 – 3.3.3). Body size and mass were highest during winter (June to August) when water temperature was at a minimum. The period of minimum size and mass corresponds with the highest water temperature over the summer months (November to March).

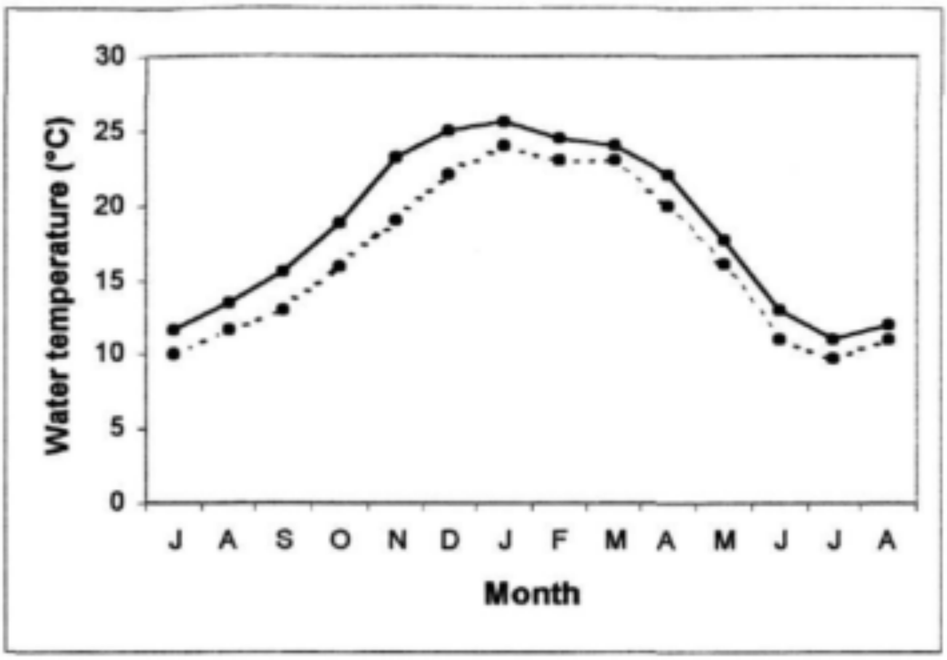


Fig. 3.2. Weekly water temperature recorded at Gifkloof (solid line) and Prieska (broken line) for the period July 1999 – August 2000.

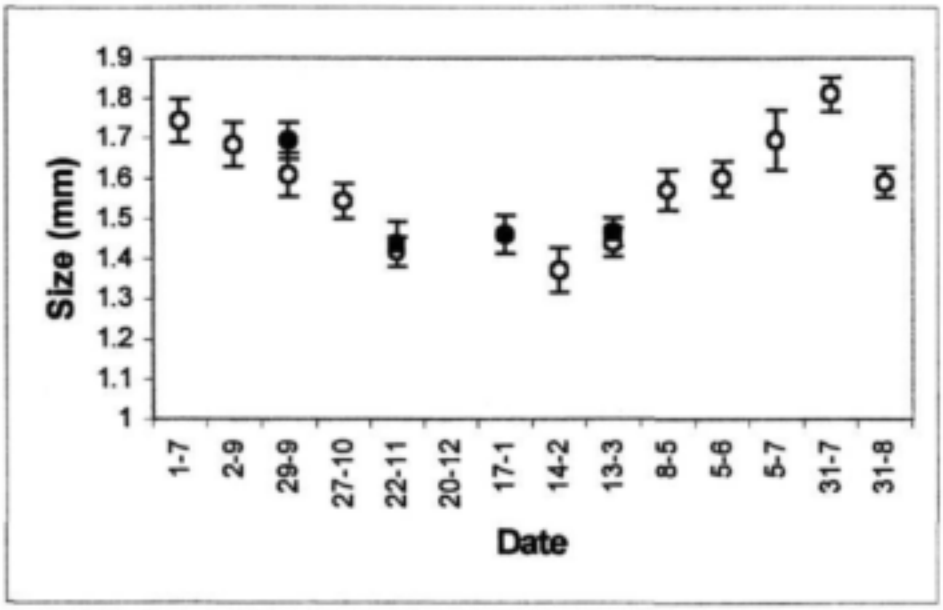


Fig. 3.3.1. Mean ± SE body size of *S. chutteri* pupae from Gifkloof and Prieska for the period July 1999 – August 2000 (Gifkloof = open symbols, Prieska = closed symbols).

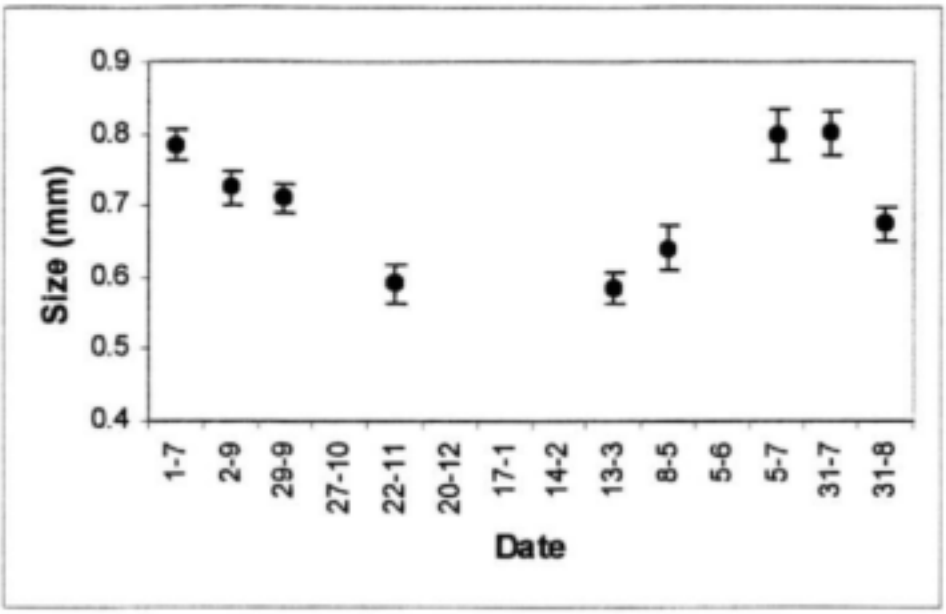


Fig. 3.3.2. Mean ± SE body size of *S. chutteri* adults from Gifkloof for the period July 1999 – August 2000.

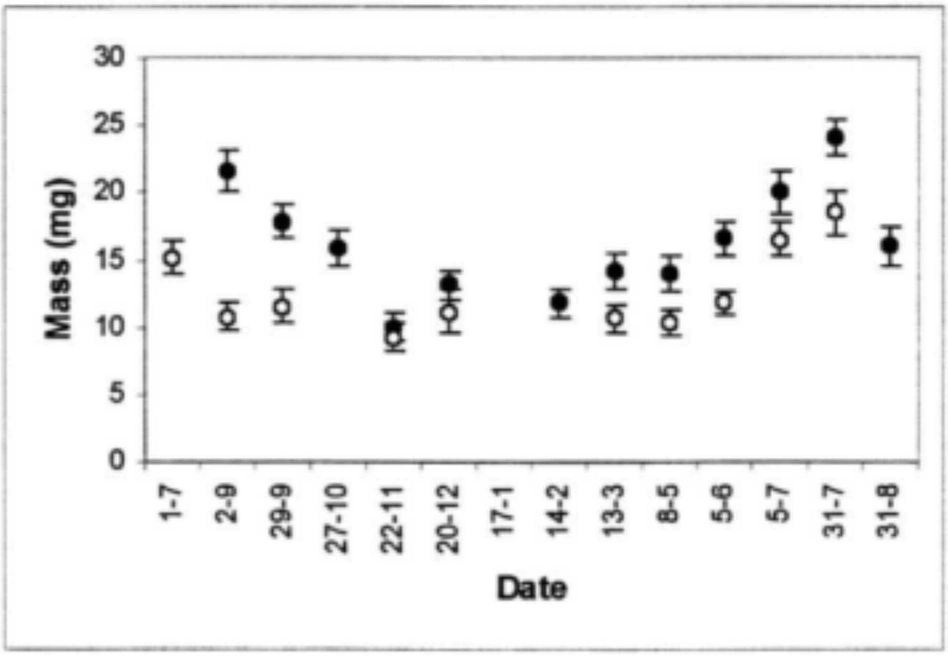


Fig. 3.3.3. Mean ± SE body mass of *S. chutteri* pupae and adults from Gifkloof for the period July 1999 – August 2000 (pupae = closed symbols, adults = open symbols).

Regression analyses of size on developmental temperature showed significant, strong inverse relationships between developmental temperature and body size in Gifkloof pupae, Gifkloof adults and Prieska pupae (Table 3.2.1, Fig. 3.4), and the same was true of body mass where this was determined (Table 3.2.2, Fig. 3.4). The ANOVA showed that Prieska pupae were significantly larger than the Gifkloof pupae ($P < 0.001$, $F = 11.13$), but when developmental temperature was included as a co-variate the site differences were no longer significant ($p = 0.23$).

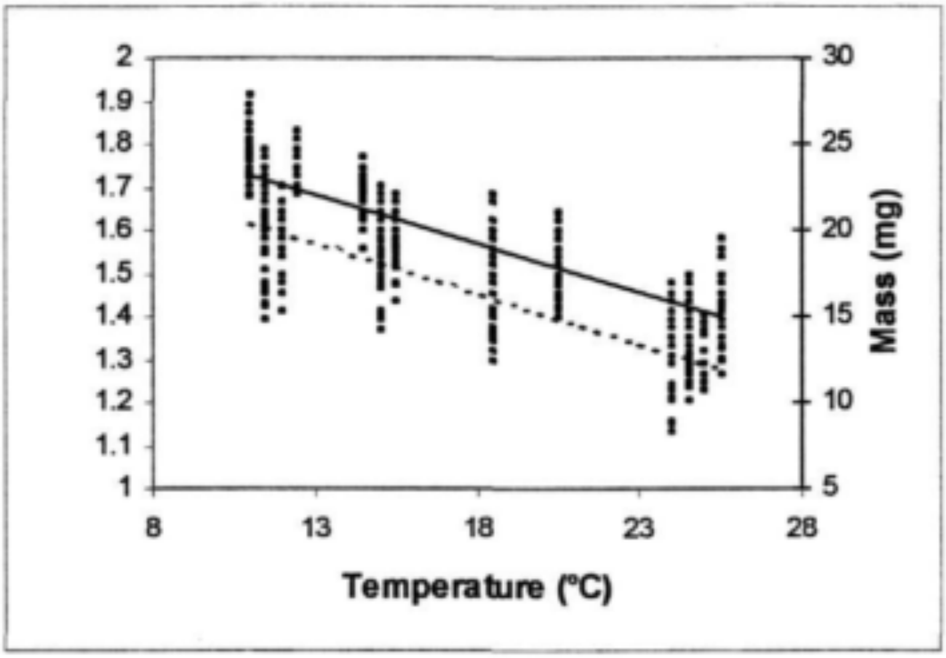


Fig.3.4. Regression plots of *S. chutteri* female pupal size (solid line) and mass (broken line) on developmental temperature.

Table 3.2.1. Regression analyses of the size of various S. *chutteri* groups on developmental temperature.

Site	*Developmental stage*	*Slope ± SE*	*Intercept ± SE*	r^2	*P*	*F*	*n*
Gifkloof	Pupae	-22.73 ± 0.60	1979.99 ± 10.68	0.71	< 0.001	1439.30	600
Gifkloof	Adults	-14.30 ± 0.47	936.00 ± 7.75	0.65	< 0.001	932.44	500
Prieska	Pupae	-24.95 ± 0.83	2017.71 ± 17.12	0.82	< 0.001	896.21	200

Table 3.2.2. Regression analyses of the mass of various S. *chutteri* groups on developmental temperature.

Site	*Developmental stage*	*Slope ± SE*	*Intercept ± SE*	r^2	*p*	*F*	*n*
Gifkloof	Pupae	-0.58 ±0.04	26.93 ± 0.81	0.61	< 0.001	187.60	120
Gifkloof	Adults	-0.44 ± 0.04	20.52 ± 0.65	0.57	< 0.001	143.47	110

3.3.3 Metabolic reserves

Regression analyses of the percentage lipid per sample on average body mass indicated that there are significant, linear relationships between these variables in both pupae and adults (Table 3.3.1, Fig. 3.5.1). The percentage lipids per sample and developmental temperature were significantly, inversely related in both pupae and adults (Table 3.3.2, Fig. 3.5.2).

Regression analyses showed no significant relationship between the percentage glycogen per sample and average body mass in either pupae or adults (Table 3.4.1, Fig. 3.5.1). In the case of percentage glycogen and developmental temperature the relationships were also not significant (Table 3.4.2, Fig. 3.5.2).

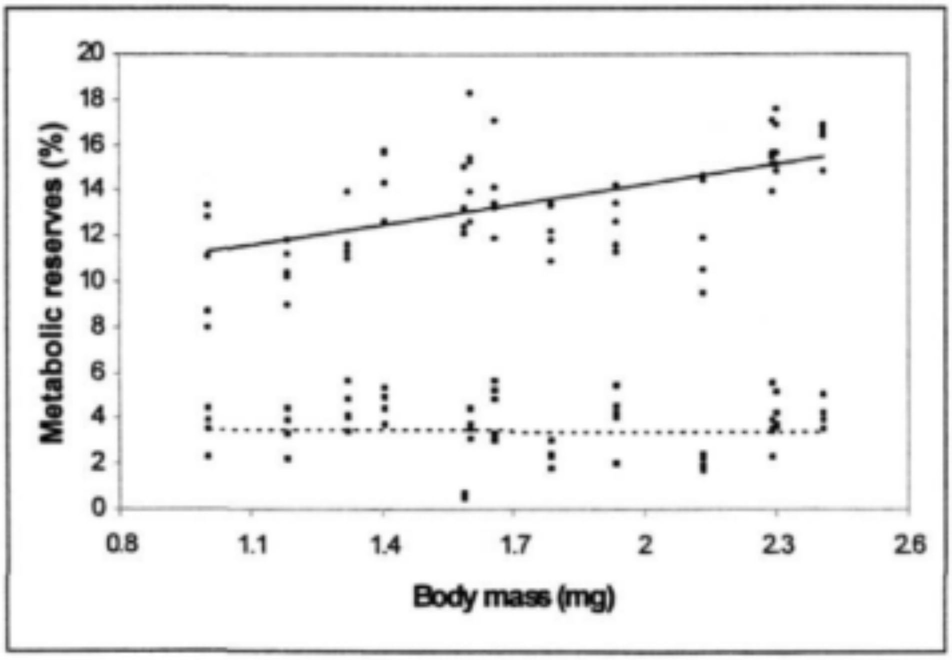


Fig. 3.5.1. Regression plots of lipid (solid line) and glycogen (broken line) content of *S. chutteri* pupae on body mass.

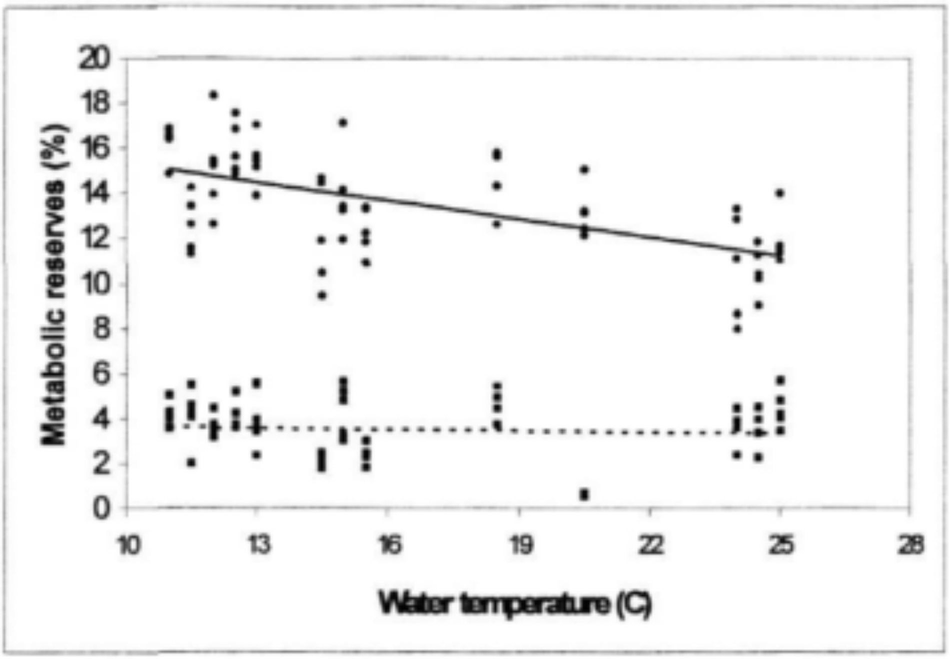


Fig. 3.5.2. Regression plots of lipid (solid line) and glycogen (broken line) content of *S. chutteri* pupae on developmental temperature.

Table 3.3.1. Summary statistics of least square linear regression analyses of body mass against percentage lipid content.

Developmental stage	*Slope ± SE*	*Intercept ± SE*	r^2	*p*	*F*	df
Pupae	2.969 ± 0.560	8.311 ± 1.004	0.31	< 0.001	28.10	64
Adults	10.839 ± 1.082	-1.149 ± 1.374	0.68	< 0.001	100.29	49

Table 3.3.2. Summary statistics of least square linear regression analyses of developmental temperature against percentage lipid content.

Developmental stage	*Slope ± SE*	*Intercept ± SE*	r^2	*p*	*F*	df
Pupae	-0.274 ± 0.048	18.052 ± 0.829	0.35	< 0.001	33.21	64
Adults	-0.465 ± 0.066	20.252 ± 1.181	0.51	< 0.001	49.92	49

Table 3.4.1. Summary statistics of least square linear regression analyses of body mass against percentage glycogen.

Developmental stage	r^2	*p*	*F*	df
Pupae	0.001	0.80	0.06	64
Adults	0.004	0.97	0.002	49

Table 3.4.2 Summary statistics of least square linear regression analyses of developmental temperature against percentage glycogen.

Developmental stage	r^2	*p*	*F*	df
Pupae	0.007	0.52	0.42	64
Adults	0.022	0.30	1.08	49

3.4 Discussion

Body size and mass of *S. chutteri* pupae and adults vary seasonally and are strongly associated with changes in developmental temperature. In all the groups studied both body size and mass reached a maximum during mid-winter (June – August) when water temperature at both study sites was lowest. Correspondingly, body size and mass reached a minimum during summer (November – March) when water temperature was highest. These seasonal changes in size are similar to that reported for *S. chutteri* larvae by Palmer et al (1995b) along the Orange River and by De Moor (1982a) along the Vaal River. In contrast to the work undertaken here, these authors provided no data on developmental temperature, but the present study confirms previous suppositions regarding temperature-related seasonal changes in the body size of *S. chutteri* recorded by them. Similar seasonal variations in size have been recorded for blackflies species from around the globe (Chutter, 1970; Ross and Merritt, 1978; De Moor, 1982a; Merritt et al, 1982; Baba, 1992; Hadi and Takaoka, 1995).

Regression analyses showed that the seasonal body size and mass changes are strongly correlated with water temperature. This pattern is typical of that expected for small ectotherms (Atkinson, 1994), and particularly those where growth can take place throughout the season (Chown and Gaston, 1999). These results also provide support for previous laboratory studies done on simuliids, which showed that temperature has a considerable effect on final adult (and pupal) body size (Colbo and Porter, 1979; 1981). However, these correlations only suggest a causal link between temperature and body size, and do not constitute evidence for it. Indeed, several studies have shown that other environmental factors such as parasitism (Colbo, 1982; Crosskey, 1990), crowding (Colbo and Porter, 1979; Colbo, 1982), nutrition (Chutter, 1970; Colbo and Porter, 1979; 1981; Scriber and Slansky, 1981; Crosskey, 1990) and hydrological conditions (Hauer and Benke, 1987) also vary seasonally and can influence body size in simuliids. The present demonstration, however, shows that significant differences in body size associated with geography (i.e. between Prieska and Gifkloof, which differ in temperature) can be removed if water temperature is included as a covariate in the model, strongly suggests that temperature plays a key role in determining adult body size in *S. chutteri*. This provides considerable support for authors such as De Moor (1982a) who proposed that developmental temperature is the proximate factor affecting the size of *S. chutteri*. Of course, the potential influence of other environmental factors cannot be ignored, and some of the residual variance found within the samples is undoubtedly a consequence of these factors.

The maximum size obtained by *S. chutteri* at the coldest temperatures is strongly related to the period of longest development given by Palmer (1997). In turn, minimum size is related to the periods of shortest development time. This pattern has also been well illustrated in other blackfly species (Begemann, 1980; De Moor, 1982a; Meritt et al, 1982; Hauer and Benke, 1987; McCreadie and Colbo, 1991). According to Crosskey (1990) these changes not only occur seasonally, but also globally, and in general the rate of blackfly development increases in the warm tropics and gives rise to smaller individuals than in the cooler arctic and temperate areas.

Few previous studies have been done on the metabolic reserves of blackflies and conclusions have mostly been drawn from studies of fecundity. These studies generally show that increased size is associated with increased fecundity, which in turn is dependent on increased lipid and glycogen contents (Chutter, 1970; Crosskey, 1990). In the present study clear evidence was found for an association between body mass and lipid content. However, there was not only a directly proportional relationship between body mass and lipid content (as has been found in drosophilids and mosquitoes, where individuals developing at colder temperatures have greater lipid reserves at emergence than those developing at warmer temperatures (Chutter, 1970; Colbo, 1982; Crosskey, 1990)), but a proportionately greater lipid content in larger individuals. Thus, larger individuals have considerably greater lipid reserves than smaller ones. Such a disproportionate increase in reserves in larger individuals, and the fitness advantages thereof, have been demonstrated in several other insect species (e.g. Lighton et al, 1994; Ernsting and Isaaks, 1997; Chown and Gaston, 1999). It is likely that disproportionately higher lipid contents in *S. chutteri* either increase survival, or, as Chutter (1970) has shown, lead to enhanced fecundity. On the other hand, glycogen content, which is used as the primary flight fuel in blackflies (Crosskey, 1990), did not show disproportionate increases. Rather, large flies have the same proportion of flight fuel as flies of a smaller size.

Therefore, it has been conclusively demonstrated here that individual *S. chutteri*, which develop at low temperatures, have a larger body size than those developing at higher temperatures. A consequence of this larger body size is disproportionately greater lipid reserves for autogenous egg production and/or survival. However, flight fuel (glycogen) reserves remained constant and appear to adequately serve the needs of individuals of a given body size. If lipid reserves result in enhanced longevity, these findings suggest that there should be pronounced seasonal variation in the longevity of blackflies, such that individuals emerging in winter, or possibly spring, will have the greatest longevity over a wide range of conditions. The following chapter sets out to test this hypothesis.

4. THE SEASONAL INFLUENCE OF TEMPERATURE, RELATIVE HUMIDITY AND STARVATION ON THE LONGEVITY OF *S. CHUTTERI*

4.1 Introduction

Previous studies on small ectotherms have shown that relative humidity (RH) and temperature are the two most important factors affecting longevity of these animals (Bursell, 1974; Parsons, 1983; Da Lage et al, 1989; Hoffman, 1990; Sawabe and Mogi, 1999). Temperature's importance stems from the fact that small ectotherms are unable to physiologically control their body temperature and thus it reflects ambient temperature. In addition, because of their small size, thermal inertia has no role in behavioural regulation (Hochachaka and Somero, 1985; Stevenson, 1985; cited by Junge-Berberovic, 1996). Similarly, relative humidity (RH) is important because small terrestrial arthropods are particularly susceptible to desiccation owing to their large surface area to volume ratio (Dwarakanath et al., 1974; Schmidt-Nielsen, 1984; Gibbs et al, 1997). Consequently, longevity declines with an increase in temperature and decrease in relative humidity (Jordan and Hubbard, 1991). Given that stress can broadly be defined as any environmental factor (or factors) that serves to reduce the fitness of an organism (Koehn and Bayne, 1989), and that longevity is an important component of fitness, it is not surprising that in ectotherms, increases in longevity are often linked to increased resistance to stresses, such as elevated temperatures and desiccation (Service et al, 1985; Chown and Gaston, 1999).

Not all populations and individuals, however, show similar levels of tolerance to such stresses. Indeed there is often substantial variation both within and between populations, and this variation is often linked to differences in metabolic rate (Service, 1987; Hoffman, 1990; Hoffman and Parsons, 1989a; b), body size (Parsons, 1970; Nevo, 1973; Barker and Barker, 1980; Clark and Doane, 1983; Chown and Gaston, 1999) and lipid and glycogen reserves (Rose, 1984; Service et al, 1985; Service, 1987; Sawabe and Mogi, 1999).

Simulium chutteri adults are not only exposed to extreme temperatures and periods of low relative humidity along the Orange River (see Chapter 2), but also have to tolerate additional stresses such as starvation (Palmer, 1997). Because the body size and lipid and glycogen contents of *S. chutteri* vary seasonally (see Chapter 3), and because it has been demonstrated in other species that longevity is related to size and the quantity of metabolic reserves, there should be seasonal variation in the longevity of this species. This, in turn, might have significant consequences for population build-up as a consequence of longer periods available for egg-laying in females, and an increased ability of the flies to overcome potentially stressful conditions. Here, the hypothesis that temperature-driven variation in body size and metabolic reserves influences longevity, is tested. This was done by examining the longevity of flies, which emerged in each of the four major seasons, under a range of temperature and relative humidity regimes.

4.2 Materials and methods

4.2.1 Experimental design

Prior to the onset of the longevity trials critical thermal trials were conducted to determine the temperature extremes that *S. chutteri* can tolerate, and to calculate the appropriate temperatures for use in the longevity trials. To take seasonal variation into account, critical thermal trials were performed during mid-summer (December 1999) and mid-winter (June 2000) using a modification of the method described by Klok and Chown (1998), but which was similar to the technique described by Huey et al (1992).

Ten newly emerged, unfed blackfly adults of mixed sexes were placed inside a "Huey-Chamber" connected to a Grant LTD 6 water bath (0.1 °C accuracy) with a PZ1 programmable temperature controller. A 40 gauge copper-constant thermocouple, connected to a Kane May 457XP thermocouple, was placed inside the Huey-Chamber to measure the ambient temperature.

The mid-summer population was acclimatized at 23 °C for 10 minutes and then the temperature was lowered at 0.5 °C.min^{-1}. The temperature at which each fly lost its righting response was recorded as the critical thermal minimum onset ($CT_{min/o}$) for that individual. The temperature was then lowered at the same rate to 0° C, and was held there for five minutes. The temperature was then increased at a rate of 0.5 °C.min^{-1} until each fly regained its righting response. This temperature was recorded as the critical thermal minimum recovery ($CT_{min/r}$) for each individual. For critical thermal maxima (CT_{max}) the temperature was increased at 0.5 °C.min^{-1} until each fly lost complete locomotor function.

For the winter trials the same procedures were followed except that the flies were acclimatized at 12 °C and the temperature was decreased to –2 °C. All trials were repeated five times and the means and standard deviation (SD) were calculated for each group (Table 4.1).

The critical thermal trials showed that the CT_{max} of both *S. chutteri* populations was *c.* 43 °C and therefore 38 °C was selected as the upper limit for the longevity trials. Both $CT_{min/o}$ and $CT_{min/r}$ showed considerable between-season variation (Table 4.1), but because the $CT_{min/o}$ of the summer population was highest (7.0 ± 0.8 °C), 10 °C was chosen as the lower temperature limit for the longevity trials. Longevity trials were also conducted at two temperatures (20 and 30 °C) between these extremes.

Table 4. 1. Summary statistics for $CT_{min/o}$, $CT_{min/r}$ and CT_{max} of winter and summer adult *S. chutteri*.

	Population	**Mean ± SD**	**Max**	**Min**	**n**
$Ct_{min/o}$	Summer	7.0 ± 0.8	8.8	5.6	54
	Winter	0.7 ± 0.9	2.8	-0.7	66
$Ct_{min/r}$	Summer	8.4 ± 1.1	10.4	6.2	54
	Winter	2.6 ± 1.3	5.1	0.2	65
CT_{max}	Summer	43.4 ± 0.9	44.5	40.2	54
	Winter	42.7 ± 1.3	45.1	39.4	54

At each of these four temperatures, longevity was assessed at four different relative humidities selected to represent the full scale (i.e. 0, 33, 75 and 100 %). Humidities were controlled by keeping flies in custom-made chambers. A chamber consisted of a 450 ml transparent plastic container and a lid. The lower half of the container was sealed off with gauze and was used to house the humidity controlling substance, whereas flies had access to the upper half of the chamber only. Relative humidities of 0 and 100 % were obtained by placing silica gel and distilled water, respectively, in humidity controlled chambers. Humidities of 33 and 75 % were obtained by respectively using saturated $MgCl_2.6H_2O$ and NaCl solutions in the humidity controlled chambers (see Winston and Bates, 1960). The trials were repeated four times, commencing on 10 July 2000 (Winter population), 10 October 2000 (Spring population), 10 January 2001 (Summer population), and 8 April 2001 (Autumn population).

For each trial, 970 females were hatched from Gifkloof pupae as described in Section 3.2.1. Of these, 50 were used for size measurements, 100 for mass determination (as described in section 3.2.3) and 20 for the determination of the lipid and glycogen contents (as described in section 3.2.4). The 800 remaining specimens were used in the longevity trials.

On emergence, flies were placed individually in 1.5 ml eppendorf tubes, and for feeding purposes, were given access to a cotton ball soaked in 20 % sucrose. Flies were fed because previous studies on simuliids showed that adults in the laboratory survive for only 1 – 2 days without a sugar meal (Rodriquez-Perez et al, 1995). In addition, the availability of a sugar meal is likely to represent natural circumstances, because flies generally have access to nectar sources year-round (Myburgh et al, 2002). During the feeding period flies were placed in a refrigerated incubator at 20 ± 1 °C for 12 hours at constant light.

After feeding, flies were placed in groups of 10 in the humidity controlled chambers. The chambers were sealed immediately and only reopened after 100 % mortality. For each temperature and humidity, five chambers with 10 specimens each were used. Incubators were kept at a 12:12 L:D cycle. The number of dead flies in each container was counted at regular intervals, which ranged between hourly and daily observations, depending on their potential survival period. Time of death was considered the mid-point between two observations.

4.2.2 Statistical analyses

4.2.2.1 Size, mass, lipid and glycogen analyses

The mean size, mass, lipid and glycogen content of each population was calculated as described in Chapter 3. To determine if the size, mass, lipid and glycogen contents differed significantly between populations, ANOVA's with multiple range tests were done on each of these variables. These analyses were done with STATISTICA (Statistica Statsoft, 1991).

4.2.2.2 Analyses of longevity data

For each test group a least-squares linear regression analysis with the number of mortalities over time were done and the resulting equation was used to calculate the LT_{50} and LT_{95} for each group. Variation in LT_{50} with temperature and relative humidity of each group was also assessed using contours derived from a least-squares model (Statistica Statsoft, 1991). To test if there were significant differences in survival, four temperature/humidity treatments were selected, based on inspection of the plots, and the data from each of these subjected to one way ANOVAS to determine the effect of season on LT_{50} and LT_{95} at these particular temperatures and humidities.

4.3 Results

The mean length, mass, and lipid and glycogen contents of the four *S. chutteri* groups used in the longevity trials are provided in Table 4.2. The winter population had the largest mean size ($F_{(3, 196)} = 514.65$, $p < 0.0001$, Tukey HSD test) and mass ($F_{(3, 36)} = 308.02$, $p < 0.0001$, Tukey HSD test), and the highest lipid ($F_{(3, 16)} = 33.01$, $p < 0.0001$, Tukey HSD test) and glycogen contents ($F_{(3, 16)} = 15.70$, $p < 0.0002$, Tukey HSD test) of all the populations tested (see also Chapter 3). The spring population had the second highest size, mass, lipid and glycogen values of all the populations tested, and size and metabolic reserves declined to summer, and then increased slightly in the autumn.

The mean LT_{50} and LT_{95} values of the various populations are presented in Table 4.3. They show that longevity in all the groups decreased as temperature increased and humidity decreased. Furthermore, a dramatic increase in longevity can be seen in all the groups when temperatures were lowered to 10 °C and RH increased to 100 %. The longevity plots of the four populations are shown in Fig. 4.2.

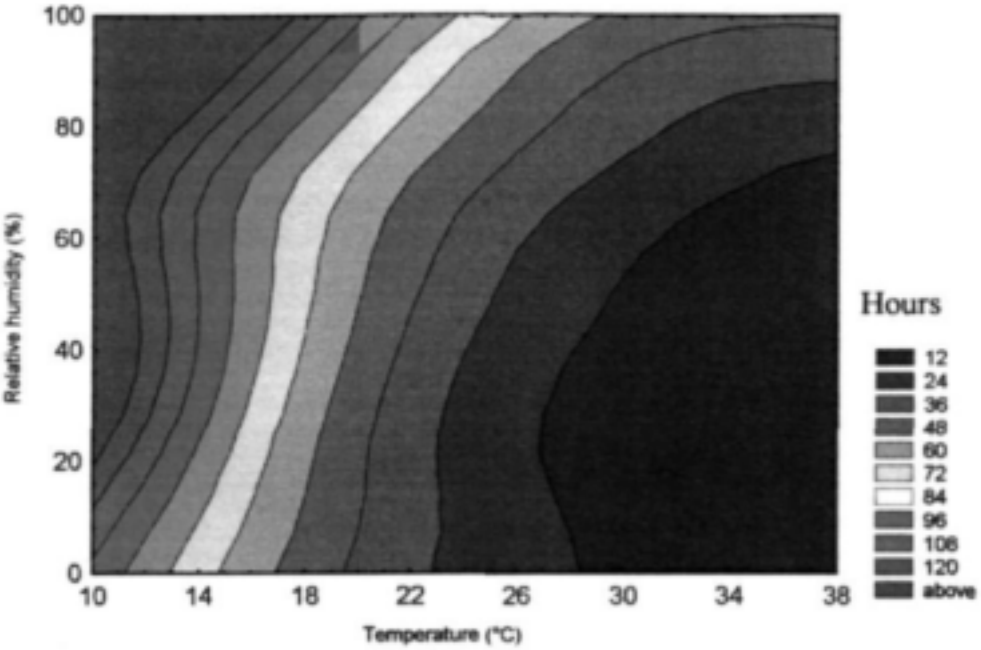
Table 4.2. Mean size, mass, lipid and glycogen content of female *S. chutteri* used in the longevity trials. Sample size is shown in brackets.

Date	Season	Size ± SE (µm)	Mass ± SE(mg)	Lipids ± SE (mg/mg)	Glycogen ± SE (mg/mg)
10/7/2000	Winter	1743.98 ± 4.72 (50)	20.12 ± 0.35 (10)	14.12 ± 0.65 (5)	5.21 ± 0.27 (5)
10/10/2000	Spring	1586.94 ± 6.91 (50)	16.23 ± 0.14 (10)	12.33 ± 0.42 (5)	4.19 ± 0.19 (5)
10/1/2001	Summer	1372.38 ± 8.33 (50)	11.17 ± 0.17 (10)	10.29 ± 0.21 (5)	3.75 ± 0.09 (5)
8/4/2001	Autumn	1461.41 ± 7.95 (50)	14.09 ± 0.12 (10)	11.79 ± 0.29 (5)	4.03 ± 0.08 (5)

Table 4.3. Mean LT_{50} and LT_{95} data (h) of *S. chutteri* females in various groups kept at various temperatures and relative humidities.

Temp (°C)	RH (%)	Populations							
		Winter		Spring		Summer		Autumn	
		LT_{50} ± SE (h)	LT_{95} ± SE (h)	LT_{50} ± SE (h)	LT_{95} ± SE (h)	LT_{50} ± SE (h)	LT_{95} ± SE (h)	LT_{50} ± SE (h)	LT_{95} ± SE (h)
10	0	88.7 ± 7.4	173.7± 19.8	167.7 ± 9.1	283.8 ± 17.8	113.5 ± 8.7	202.2 ± 11.8	113.2 ± 10.3	222.1 ± 23.8
	33	141.2 ± 9.5	256.6 ± 16.3	225.5 ± 14.3	391.8 ± 22.1	149.0 ± 23.0	293.2 ± 48.2	163.7 ± 9.4	287.3 ± 14.9
	75	133 ± 38.6	239.5± 58.9	206.9 ± 22.1	339.1 ± 38.2	164.1 ± 20.4	285.2 ± 40.3	182.1 ± 2.3	299.4 ± 5.1
	100	216.1 ± 32.2	394.8 ± 59.4	345.3 ± 20.3	559.2 ± 44.2	442. 7 ± 7.8	734.6 ± 15.5	493.5 ± 54.1	814.4 ± 89.9
20	0	31.7 ± 1.3	56.0 ± 2.7	46.5 ± 2.0	80.5 ± 1.5	36.4 ± 6.9	67.2 ± 13.3	41.9 ± 2.2	73.7 ± 6.2
	33	29.3 ± 2.9	50.6 ± 4.3	66.0 ± 5.1	108.1 ± 9.8	33.5 ± 3.1	64.3 ± 6.1	33.2 ± 3.5	55.8 ± 5.7
	75	50.6 ± 7.2	86.8 ± 12.0	25.5 ± 6.4	44.8 ± 11.5	75.6 ± 2.9	129.1 ± 4.9	72.3 ± 8.2	122.9 ± 12.2
	100	93.8 ± 13.9	165.6 ± 21.6	140.5 ± 7.6	231.4 ± 13.4	107.2 ± 11.8	181.8 ± 17.4	108.1 ± 13.9	182.8 ± 13.9
30	0	15.2 ± 0.4	29.5 ± 1.2	21.2 ± 1.1	38.8 ± 2.0	15.8 ± 0.8	32.7 ± 1.7	19.3 ± 0.7	33.9 ± 1.2
	33	16.9 ± 1.7	30.5 ± 2.7	14.7 ± 0.6	11.9 ± 0.6	14.5 ± 1.0	26.9 ± 1.3	12.1 ± 1.0	21.8 ± 1.5
	75	38.7 ± 4.9	67.8 ± 8.0	28.8 ± 1.3	31.0 ± 0.9	28.9 ± 2.2	51.0 ± 4.5	41.3 ± 2.6	74.1 ± 6.1
	100	46.5 ± 4.9	79.7± 8.3	37.9 ± 1.1	61.7 ± 1.7	61.3 ± 5.7	101.2 ± 10.5	44.1 ± 5.7	75.8 ± 11.2
38	0	8.1 ± 0.5	19.0 ± 0.9	5.2 ± 0.4	9.5 ± 0.3	4.9 ± 0.5	10.5 ± 0.3	10.6 ± 0.6	21.1 ± 0.9
	33	4.5 ± 0.5	12.6 ± 2.3	11.3 ± 0.8	21.6 ± 0.8	8.6 ± 0.4	17.0 ± 1.2	6.4 ± 0.3	14.2 ± 0.6
	75	6.1 ± 0.3	21.1 ± 1.3	14.2 ± 0.7	32.2 ± 1.9	9.3 ± 0.4	22.5 ± 1.3	12.0 ± 0.6	21.3 ± 0.8
	100	37.5 ± 4.3	63.0 ± 2.8	14.0 ± 2.4	27.9 ± 1.1	27.6 ± 1.5	48.4 ± 3.0	17.4 ± 1.1	43.8 ± 3.6

Winter population



Spring population

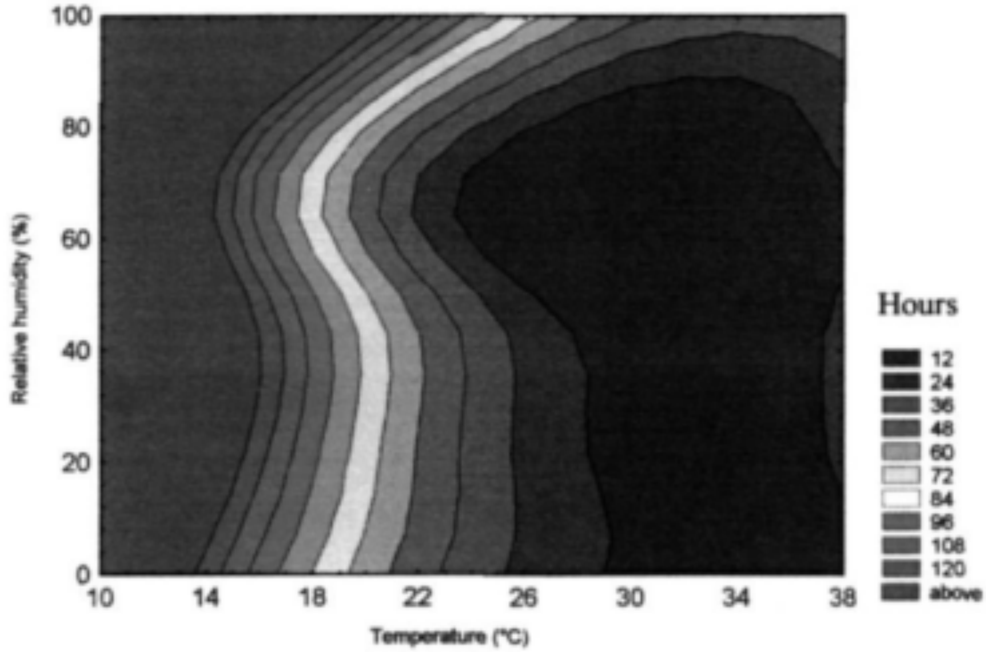
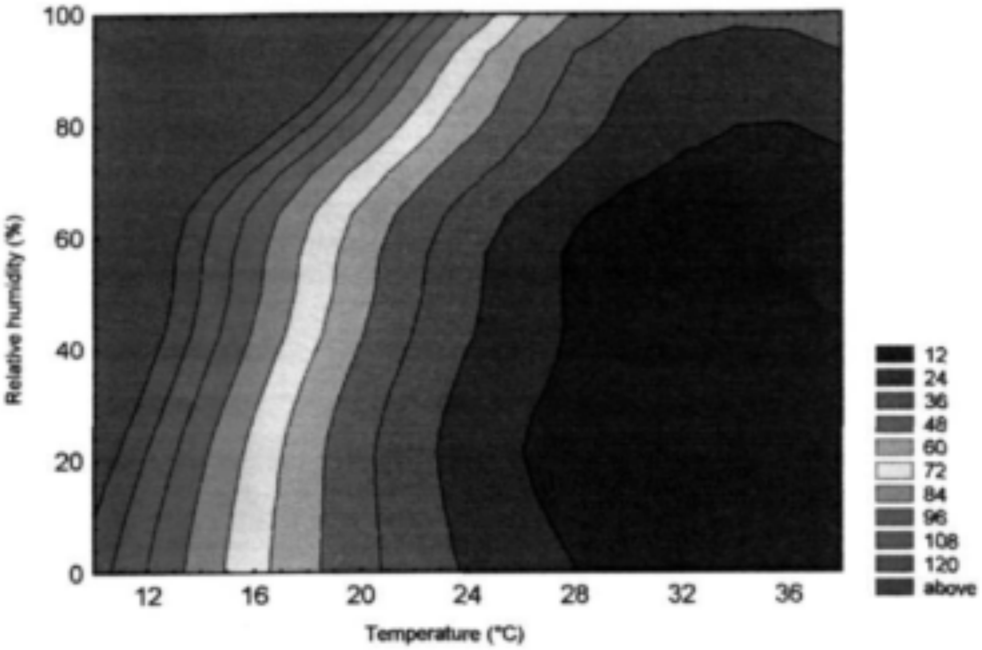


Fig. 4.2. Longevity plots of various *S. chutteri* female populations.

Summer population



Autumn population

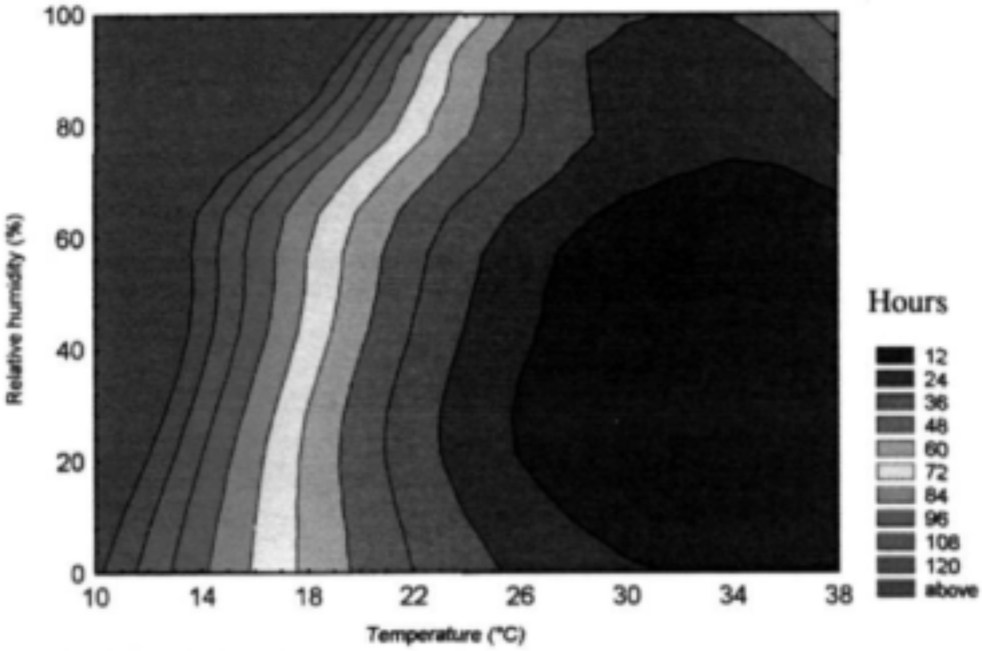


Fig. 4.2. Continued

From the longevity plots presented in Fig. 4. 2 it is clear that there were substantial variations in longevity between the various populations. In all of the populations there were decreases in longevity as temperature increased and humidity decreased, and a dramatic increase in longevity at low temperatures and high humidity. Moreover, in all the populations the contours tended to be spaced further apart as temperature increased. This indicates that the time to death was accelerated as temperature increased and therefore longevity decreased exponentially with an increase in temperature. Although a slight increase was seen in longevity when RH was increased from 0 to 60 %, the effect was more dramatic at RH exceeding 60 %. Therefore, longevity also increased exponentially with an increase in RH. These results show that the optimum conditions for survival are low temperatures and high humidities, while low RH and high temperatures severely hamper survival in all the populations.

The spring population was more sensitive to high temperatures than the winter population, although a slight increase in survival could be seen when RH was increased at the high temperatures. The spring population showed much longer survival over the rest of the conditions tested and survived considerably longer at lower temperatures. Although the spring population showed similar exponential survival times with an increase in temperature and RH, the longevity at 75 % RH appears to be lower than at 33 %. This is due to the low LT_{50}'s recorded for the spring population at 75 % RH. This can be attributed to possible contamination of this group with NaCl during the longevity trials.

The summer population appeared to be less sensitive to high temperatures than the spring population, but more sensitive than the winter population. Over the rest of the conditions tested they showed lower survival than the spring population, but higher than the winter population. The longevity plot of the autumn population is remarkably similar to that of the summer population. Although there was a similarity in the plots of the summer and autumn populations, the autumn population survived slightly longer under all the conditions tested.

Much of the variation between populations was a consequence of differences in survival at the lower temperatures and higher humidities. For example, at 10°C and 100% R.H. there was a clear increase in longevity through the seasons, where the winter population had the lowest longevity, followed by the spring, summer and autumn populations. Differences between populations adjacent in time were, however, not significant, although those separated by at least another seasons differed significantly ($F_{(3, 16)} = 13.45$, $p < 0.001$, Tukey HSD test). At 10 ^{0}C and 0 % R.H. the spring population had the largest LT50, which significantly differs from the other populations that were all the same ($F_{(3, 16)} = 14.00$, $p < 0.001$, Tukey HSD test).

By comparison, at 30 ^{0}C and 100 % R.H. the only difference was between the summer and spring populations, where the summer had a significantly larger LT_{50} than the spring population ($F_{(3, 16)} = 4.38$, $p < 0.01$, Tukey HSD test). At 30^{0}C and 0 % R.H. the winter and summer populations had the lowest LT_{50}'s and the spring and autumn populations the highest. Here the only significant differences were between adjacent populations ($F_{(3, 16)} = 23.56$, $p < 0.001$, Tukey HSD test).

Considering the fact that longevity in all the populations tested increased considerably above an RH of approximately 60 %, for discussion purposes a RH of 0 – 60 % will be regarded as low and a RH of 61 – 100 % as high. Similarly temperatures of 0 - 15 ^{0}C will be regarded as low, 16 – 25 ^{0}C as medium and 26 – 38 ^{0}C as high.

4.4 Discussion

Previous studies on simuliids have shown that longevity in this group is highly variable and generally depends on factors such as species, sex, nutritional status and weather conditions (Palmer, 1997). It is generally agreed that adult blackflies survive for only one to two weeks in nature, but under optimal conditions it has been shown that they can live for three to five weeks (Gillies, 1964). Mark-recapture studies in the Cameroon showed that females survived only 10 days, while similar studies in South America showed that most species survived for shorter than one week, but that *S. metallicum* females can survive for up to 83 days (Dalmat, 1952). However, in West Africa one female *S. damnosum* was recaptured after more than 8 months (Noamesi, 1966). Longevity studies conducted in the laboratory generally put estimates between 19 – 39 days, depending on the species involved (Raybould, 1967).

Preliminary laboratory studies done by Palmer (1997) on the longevity of *S. chutteri* showed that adult half-life ranged between a few hours when kept at 41 °C to 13 days when kept at 4 – 7 °C. He also recorded one female that lived for 22 days. Unfortunately, RH was not controlled in these trials and seasonal variation was not taken into account, but these trials nonetheless support the findings presented here, where longevity decreased with an increase in temperature. Furthermore, the present study showed that the longevity of *S. chutteri* varied between less than 2 hours under severely stressful conditions (i.e. low RH and high temperature) to more than four weeks under optimal conditions (i.e. high RH and low temperature), which is similar to the data presented by Palmer (1997).

Previous studies on small ectotherms showed that at low and moderate temperatures death usually occurs from starvation (Da Lage et al, 1989; Van Es et al, 1998). Therefore, increased survival under these conditions can be regarded as an indicator of relative starvation resistance (Van Es et al, 1998). As the winter population showed the shortest survival under these conditions it can be concluded they had the lowest starvation resistance of all populations tested. Continuing this line of reasoning the spring population showed the highest starvation resistance followed by the autumn and summer populations.

Similarly, increased survival under dry conditions (i.e. RH approaching 0 %) can be used as an indicator of desiccation resistance in small ectotherms (Parsons, 1983; Da Lage et al, 1989; Gibbs et al, 1997). Under these conditions the winter population again showed the shortest survival of all populations tested. The spring population showed the longest survival under desiccation conditions. The summer and autumn populations behaved similarly under dry conditions and showed slightly lower desiccation resistance than the spring population.

Although many physiologists agree that different stress tolerances in ectotherms are generally positively correlated with each other, body size and metabolic reserves (Parsons, 1969; Westerman and Parsons, 1973; Service et al, 1985) these correlations were not found in this study. Although the winter population had the highest mean body size, mass, lipid and glycogen reserves they showed lower resistance to stresses such as desiccation and starvation than the other populations, at low temperatures. At higher temperatures, they did not exceed the survival of the other seasonal groups, but were rather quite similar, despite their increased body size and reserves.

One possible explanation for this phenomenon can be found in the potential fecundity of the winter population. There is strong evidence that the winter population (i.e. the first gonotrophic cycle) of *S. chutteri* is autogenous (De Moor, 1982a; Palmer, 1997). Autogeny is considered a physiologically expensive process that requires a large portion of the available metabolic reserves for the development of the oocytes (Wheeler, 1996). It therefore seems likely that diverts energy and other resources away from other fitness components such as desiccation and starvation resistance. It can thus be argued that these show a negative correlation with fecundity (autogeny) in *S. chutteri*. Similar findings have been recorded in *Drosophila* literature, where negative correlations have been found between fecundity and tolerance to environmental stresses such as desiccation, ethanol and nutrition (Service et al, 1985; Service and Rose, 1985). Hoffman and Parsons (1989b) also showed that increased mating ability, fecundity and fertility might be correlated with low resistance to stress in this group.

Adaptive explanations for the decreased survival of the winter population can also be proposed. Because the winter population will be exposed to far more favourable environmental conditions than the other populations upon emergence (see Chapter 2), increased resistance to environmental stresses will not be a prerequisite for prolonged survival, and energy and other resources can thus be used for autogenous egg development. Consequently, adults do not have to go off in search of a blood meal to develop the offspring and therefore, they will not be exposed to harsh environmental conditions as they can take shelter in the drainage lines of the Orange River and its tributaries (Myburgh, et al, in press). It can therefore be argued that increased stress resistance is not ecologically important for the successful survival and reproduction of the winter population.

By comparison, environmental conditions are generally harsh over the summer and autumn periods. Therefore, increased starvation resistance may be important to the survival of the species as their activity will be limited to rather short periods (Palmer, 1997) and they will thus have to tolerate periods of starvation. When they do go off in search of a meal increased tolerance to desiccation will become important to ensure prolonged survival. Similarly it might be argued that the spring population would be exposed to more favourable environmental conditions that will allow them to seek a sugar or blood meal for longer periods than the other populations. Therefore, tolerance to stresses such as desiccation and starvation will improve their overall fitness. Thus, despite a small body size, and consequently lower reserve level (especially of lipid which will be disproportionately reduced in smaller individuals), the summer population has considerable resistance to both desiccation and starvation.

This chapter showed that that major seasonal variation occurs in the survival of *S. chutteri*, as it does in other ectotherm species (such as scorpions (Toolson and Hadley, 1979), tenebrionid beetles (Hadley, 1977) and Drosophiliidae (Hoffman, 1990)), and that longevity can not merely be attributed to environmental conditions, but that biotic factors such as potential fecundity, body size, mass and energy reserves are also important considerations. Moreover, it was shown that various trade-offs exist between these traits and that an increase in fecundity may indeed decrease the fitness of an individual by decreasing its tolerance to stress.

5. THE ROLE OF FLOWERING PLANT SPECIES IN ADULT BLACKFLY SURVIVAL

5.1 Introduction

Carbohydrates play an important role in blackfly ecology as male and female blackflies need it for flight energy and increased longevity (Hocking 1953; Davies *et al.* 1962; Hunter 1977; Sutcliffe 1986). Females of some species require carbohydrates for ovarian development (Cupp & Collins 1979) and in other species carbohydrates permit autogeny (Corbet 1964; Hunter 1977). Carbohydrates are obtained from nectar (Lewis & Domoney 1966; Hunter 1977; Brenner & Cupp 1980; Cupp 1981, Wenk 1981; Crosskey 1990), other plant juices (e.g. phloem sap) (Crosskey 1990) or homopteran honeydew (Burgin and Hunter 1997a,b,c). Since carbohydrates are primarily obtained from floral or extrafloral nectaries (Crosskey 1990), blackfly seasons are, in general, associated with the season of nectar production (Hocking 1953; Burgin and Hunter 1997a).

This may prove to be an important consideration in the survival of adult blackflies as the arid conditions along the Orange River suggests that nectar availability may be severely limited during certain periods (Palmer 1997). Furthermore, plants in arid and semi-arid summer rainfall regions have characteristic short flowering seasons. The non-flowering period for most plant species also occurs during the hot and dry summer months (Struck 1994).

However, plants are not only a source of carbohydrates, but also give blackflies protection against harsh environmental factors and predation and furthermore they provide resting sites (Lewis & Domoney 1966; Brenner & Cupp 1980; Crosskey 1990; McCreadie *et al.* 1994; Reyes-Villanueva & Rodriquez-Perez 1994). Service (1977) stressed the importance of having knowledge on the resting behaviour of insect vectors as this is important in developing protocols to study host preferences, species composition and age structure. Considering this, it is surprising to note that as recently as 1994 information on the natural resting sites of blackflies existed only as general accounts (Reyes-Villanueva & Rodriquez-Perez 1994).

The study reported on here aimed to investigate the carbohydrate availability to blackflies, their feeding preferences and resting or sheltering behaviour in plants along the lower Orange River. The knowledge obtained will be related to adult survival.

5.2 Materials and methods

5.2.1 Vegetation studies

Plant phenophases influence the availability and palatability of food to browsers, especially in arid areas (Fabricius & Van den Berg 1993). Using this principle, and applying it to flowering times, 10 individuals of each of the 29 most abundant plant species in the AFNP as recorded by Bezuidenhout (1996) were selected and permanently marked. For one year (January – December 2000) these 290 plants were individually studied at 28-day intervals for the presence or absence of leaf growth, shoot growth, flower buds, flowers, immature seeds, mature seeds, leaf yellowing and leaf abscission. A species was considered to be in a new phenophase if two or more individuals were in this phase (Hoffman 1989).

Although blackflies seem to be discriminate nectar feeders, they will feed on almost any available carbohydrate source when their preferred source is not available (Hunter 1977; Crosskey 1990). Therefore, throughout the year we also recorded flowering times of additional plant species in the AFNP. This was done by two observers who drove a fixed 26-km route during each visit and recorded all plant species that were flowering at the time. All flowering species that could be seen from the vehicle were recorded. The object of this survey was not to note any feeding activity, but only to record potential carbohydrate sources.

5.2.2 Blackfly activity

During each visit, the 290 plants were each examined for evidence of blackfly activity on flowers, and this was presumed to be feeding activity. These plants were also examined for any blackflies that could be seen resting or taking shelter. The presence of blackflies only was recorded and not their sex, species or abundance.

5.3 Results

5.3.1 Rainfall and temperature

During the present study (January - December 2000) the rainfall total for the year in the AFNP was 200mm, 5% below the average annual rainfall. Most precipitation was recorded from January to April, with the maximum monthly rainfall (95mm) in March. Light rainfall was also recorded from June – September. Average temperatures were highest from November – February and lowest from May – August (Fig. 5.1).

5.3.2 Vegetation studies

The 29 plant species studied (Table 5) showed a growth peak from January to March. A second peak was noted from September to December, although it was not as pronounced as the first. The percentage of plant species flowering throughout the year stayed remarkably consant, although small peaks were observed from January to March and during May, October and December. The percentage of plant species that carried seeds steadily decreased from a high during January to a low during July. From August onwards the percentage of plant species with seeds started to increase again.

Leaf abscission was highest during February and from June to August. During September leaf abscission decreased and increased again during October. Most plant species carried their fruit during January and February. Thereafter fruiting steadily decreased to a minimum between July and October, followed by a small peak during December (Fig.5.2).

Fig. 5.1. Total monthly rainfall (closed symbols) and average monthly temperature (open symbols) recorded in the Augrabies Falls National Park from January to December 2000.

Table 5. Alphabetic list of the 29 most abundant plant species in the Augrabies Falls National Park and the presence of blackfly feeding or sheltering activity on these plants (indicated with a "yes").

Plant species	**Feeding activity**	**Shelter**
Acacia karroo Hayne (Fabaceae)	Yes	Yes
Acacia mellifera (Vahl) Benth. subsp. *detinens* (Burch.) Brenan (Fabaceae)	Yes	Yes
Adenolobus garipensis (E.Mey.) Torre & Hillc. (Fabaceae)		Yes
Boscia albitrunca (Burch.) Gilg. & Ben. var. *albitrunca* (Capparaceae)		
Boscia foetida Schinz subsp. *foetida* (Capparaceae)		
Cenchrus ciliaris L. (Poaceae)		
Ceraria namaquensis (Sond.) Pearson & Stephens (Portulacaceae)		
Codon schenckii Schinz. (Hydrophyllaceae)		
Commiphora gracilifrondosa Dinter ex J.J.A. V.D. Walt (Burseraceae)		
Diospyros lycioides Desf. subsp. *lycioides* (Ebenaceae)		Yes
Dyerophytum africanum (Lam.) Kuntze (Plumbaginaceae)		
Enneapogon scaber Lehm. (Poaceae)		
Euclea pseudebenus E.Mey. ex A. DC. (Ebenaceae)		
Euphorbia gregaria Marioth (Euphorbiaceae)		
Ficus cordata Thunb. subsp. *cordata* (Moraceae)		Yes
Hibiscus elliottiae Harv. (Malvaceae)		
Hibiscus engleri K. Schum. (Malvaceae)		
Monechma spartioides (T. Anders) C.B. Cl. (Acanthaceae)		
Pappea capensis Eckl. & Zeyh. . (Sapindaceae)	Yes	Yes
Phragmites spp. (Cav.) Steud (Poaceae)		Yes
Rhus pendulina Jacq (Anacardiaceae)		Yes
Rhus populifolia E.Mey. ex Sond. (Anacardiaceae)		
Sarcostemma viminale (L.) R. Br. (Asclepiadaceae)		
Schotia afra (L.) Thunb. var. *angustifolia* (E.Mey.) Harv. (Fabaceae)	Yes	Yes
Sisyndite spartea E.Mey ex Sond. (Zygophyllaceae)	Yes	Yes
Stipagrostis hochstetteriana (Beck. Ex Hack.) De Winter var. *secalina* (Henr.) De Winter (Poaceae)		
Stipagrostis uniplumis (Licht.) De Winter var. *uniplumis* (Poaceae)		
Tamarix usneoides E.Mey. ex Bunge (Tamaricaceae)	Yes	Yes
Ziziphus mucronata Willd. subsp. *mucronata*. (Rubiaceae)	Yes	Yes

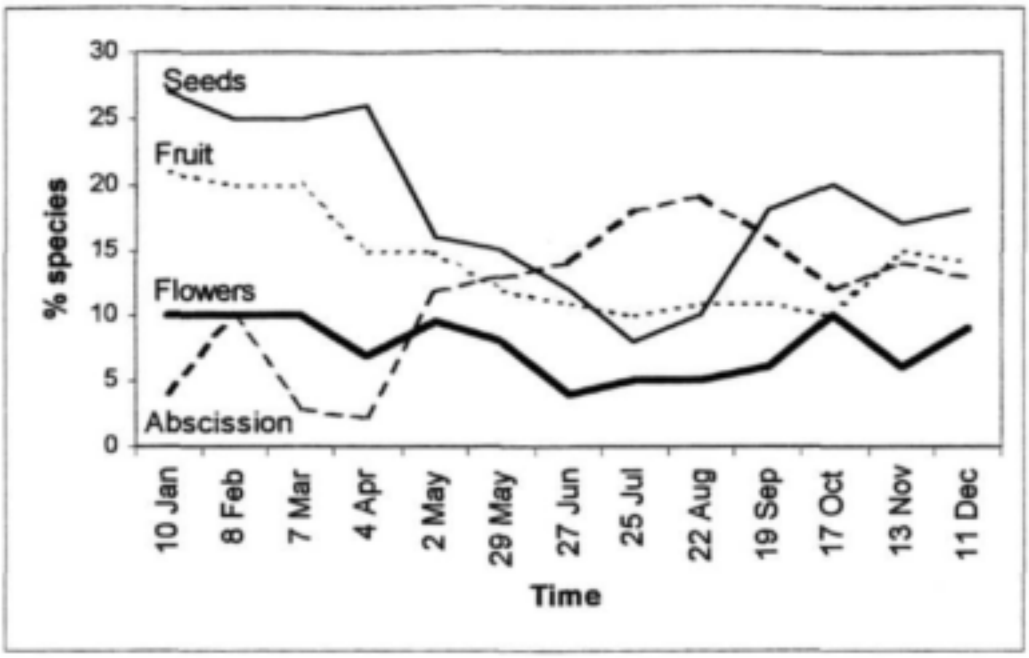


Fig. 5.2. Combined phenology of the 29 most abundant plant species in the Augrabies Falls National Park from January to December 2000.

In addition to the 29 selected plant species, a total of 66 other flowering species were recorded in the AFNP during the study period. Many of these species flowered from January to May. The number of flowering species was low for the remainder of the year, although a small peak was recorded during September (Fig. 5.3).

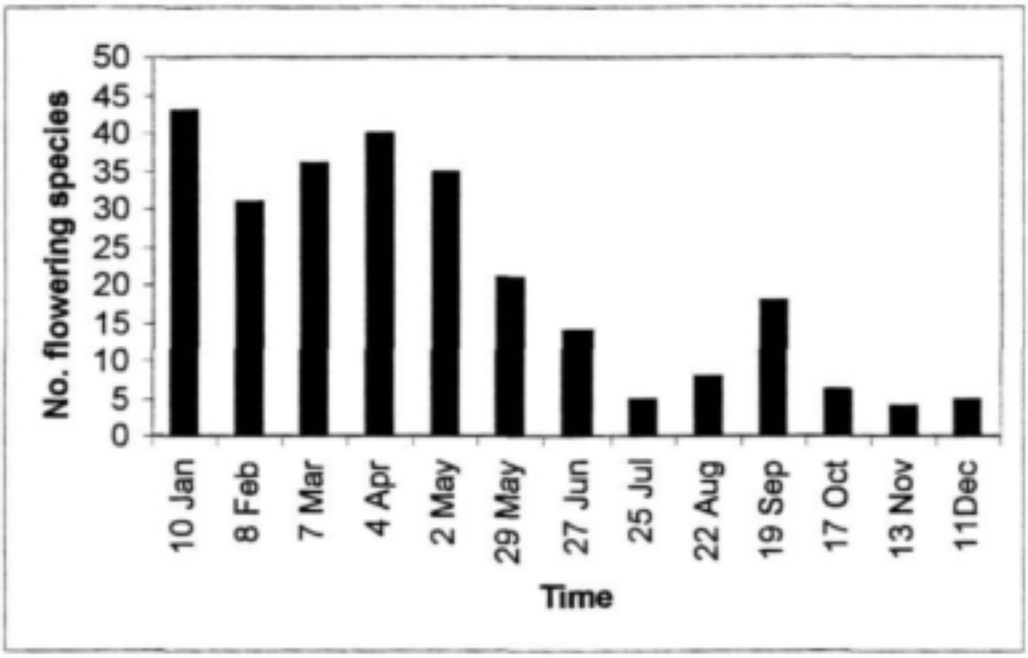


Fig. 5.3. Number of plant species flowering throughout the year in the Augrabies Falls National Park (excluding the 29 most abundant species).

5.3.3 Blackfly activity

Blackfly activity was recorded on the flowers of *Pappea capensis*, *Acacia karroo*, *Tamarix usneoides*, *Acacia mellifera*, *Ziziphus mucronata*, *Schotia afra* and *Sisyndite spartea* (Table 5). Activity was more common on the first five species and they are therefore likely to be the preferred nectar sources. At least one of these species was flowering at any given time of the year (Fig. 5.4).

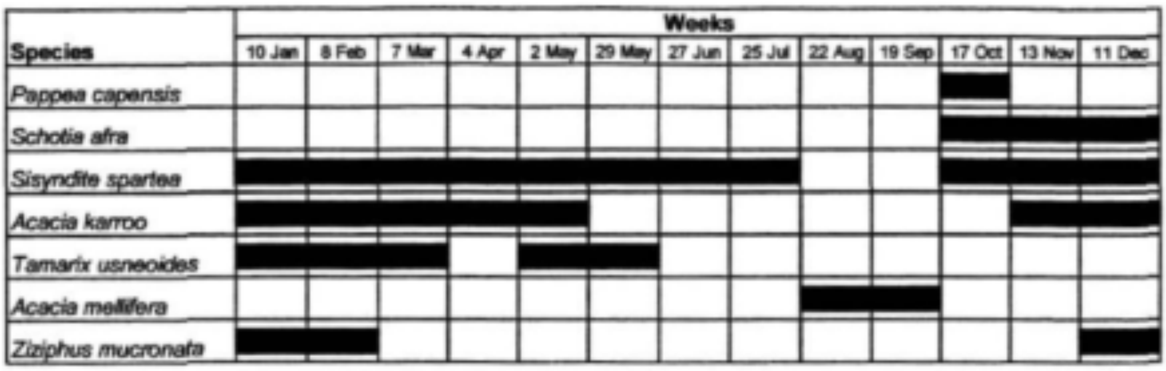


Fig. 5.4. Flowering times of the seven plant species on which blackfly feeding activity was recorded.

Blackflies did not seem to have specific preferences regarding plant species used as shelters or resting sites, although they were more common on dense shrubs and trees and were never recorded on any grass species, except the reed, *Phragmites* spp. Resting behaviour was recorded on the seven species mentioned above, and on *Adenolobus garipensis*, *Phragmites* spp., *Diospyros lycioides*, *Ficus cordata* and *Rhus pendulina* (Table 5).

5.4 Discussion

Water is the dominant controlling factor for biological processes in arid and semi-arid areas (Noy-Meir 1973; Milton 1987) and therefore the phenology of plant species in these areas is characterized by a pulse-activity response (Noy-Meir 1973). This was well demonstrated during this study where the combined phenology of the 29 selected plant species showed growth pulses after periods of rainfall. The total number of plant species (excluding the above-mentioned 29 species) flowering throughout the year was also highest after the heavy rainfall experienced early in the year. As blackflies will probably visit any angiosperm in flower (Crosskey 1990), rainfall becomes an important consideration in the survival of adult blackflies in the arid areas along the Orange River. Since more carbohydrate sources are available after periods of rainfall, the adults will not only have a greater variety to choose from, but flowers will also be more abundant.

Begemann (1986) reported visits by *Simulium chutteri* to the following plant species along the Vaal River: *Acacia karroo*, *A. mellifera*, *Ziziphus mucronata*, *Gomphostigma virgatum*, *Oenothera erythrocephala* and *Senecio burchelli*. Our study correlates well with this study where the first three species were also identified as food sources. The combined results of these two studies thus bring the total number of plant species, used as carbohydrate sources by adult blackflies in South Africa, to 10.

With the exception of *Acacia mellifera* and *Pappea capensis*, the distribution of all nectar sources used by blackflies in the AFNP is largely confined to the drainage lines of the Orange River and its tributaries. These tributaries and drainage lines are generally characterized by high canopy cover compared to the surroundings (Bezuidenhout 1996). Within these plant communities temperatures will be lower and humidity higher than experienced in adjacent vegetation. These are important considerations in adult blackfly survival, because newly emerged adults seek refugia near their breeding sites where they remain for a few hours to allow sclerotization of the integument, maturation of organs, and so forth (Reyes-Villanueva & Rodriguez-Perez 1994). The majority of female blackflies take a sugar meal prior to a blood meal (Walsh & Garms 1980; McCreadie *et al.* 1994) and male blackflies stay close to their breeding sites (Crosskey 1990). Therefore, these plant species within the drainage lines are the ideal nectar sources and shelters for newly-emerged flies. This furthermore suggests that the vegetated drainage lines may be the means by which female blackfly survive dispersal of up to 80km away from the Orange River, and also perhaps how they survive the return trip to oviposit in the same river, the only available breeding site in the area.

Female blackflies in search of a host or oviposition site can exhaust a sugar meal during prolonged flight (Crosskey 1990). They will therefore need to replenish their sugars on plant species other than those described here. In the open veld, away from drainage lines, there are fewer large bushes which may serve as nectar sources and shelter. The main, if not only species, which falls in this category and which was found in the present study to be favoured by blackfly adults, is *Acacia mellifera*. Its flowering period is restricted to two months, August and September, when adult blackflies usually emerge in high numbers from the river (Palmer 1997). *Acacia mellifera* may contribute significantly to the survival of blackflies, which disperse randomly across open ground, at this time of the year. Clearly other plant species will be used at other times of the year and therefore a similar study should be conducted on plant species in the 5 – 80km zone away from the river. The role of cultivated plant species should also be investigated. However, because of the arid nature of this area cultivated plant species will be restricted to irrigated sections along the river.

It is concluded that carbohydrate scarcity is not a limiting factor to the survival of adult blackflies along the lower Orange River, because there is always at least one of the preferred carbohydrate sources flowering at any time of the year. This hypothesis is strengthened by the fact that many other plants, that can potentially be used, flower throughout the year. The significance of drainage lines and tributaries in the survival of blackflies was shown in this study. They serve as places to rest and shelter and possibly as a means of navigation.

6. THE ROLE OF SELECTED CLIMATOLOGICAL FACTORS ON ADULT BLACKFLY ACTIVITY

6.1 Introduction

Sutcliffe (1986) proposed that blackfly activity is controlled by a single endogenous oscillator whose unimodal effect is dampened by other climatological factors that reach their extremes around midday. Many authors have provided findings that support this hypothesis and confirmed that light intensity is the primary stimulus (or oscillator) for activity in mammophilic blackflies (Steenkamp, 1972; Alverson and Noblet, 1976; Wenk, 1981; Fredeen and Mason, 1991). The effect of light intensity is, however, dampened by other, secondary climatological factors such as temperature, wind speed and relative humidity (R.H.) when these reach certain extremes (Steenkamp, 1972; Alverson and Noblet, 1976; McCreadie et al, 1986; Crosskey, 1990; Fredeen and Mason, 1991). Therefore, most blackfly species in the temperate areas exhibit a bimodal activity pattern with an increase in activity usually observed during early morning and late afternoon and a decrease in activity during midday when the secondary climatological factors limit activity (Collins et al, 1981; Crosskey, 1990). Proof for the secondary nature of the latter factors is given by authors such as Crosskey (1990) who showed that blackfly activity is initiated by light intensity, but that a decrease in activity does not occur at midday, when conditions remain favourable (i.e. calm, overcast and humid).

It is known that *S. chutteri* also exhibits a bimodal activity pattern similar to that described above (Jordaan and Van Ark, 1990; Palmer, 1997). However, no detailed study has ever been done on the specific impact of temperature, light intensity, wind speed and R.H. on the activity of this species, and consequently on the effect of these climatological factors on blackfly annoyance levels. Therefore, the objective of the current study was to describe the effect of these climatological factors on the activity levels of *S. chutteri* to be able to more accurately predict the effect of outbreaks.

6.2 Materials and methods

6.2.1 Experimental design

These trials were conducted on the farm "Swemkuil" situated approximately 80 km east of Upington and 20 km from the Orange River. The farm has a known history of blackfly attacks (L. Kotzé, pers. comm). A total of 14 trials were done from October 1999 – August 2001 (Table 6.1). Trials were conducted as follows: For the purpose of evaluating adult numbers a vehicle-mounted, human-baited trap was developed. The trap consists of a gauze net suspended from a steel structure mounted on the back of a bakkie (Fig. 6.1). The trap measures 1.8 m in height, 1.2 in width and 1.4 m in length. When set, the net is pulled to a height of 0.8 m to show a person seated on a chair. On trial dates the trap was set every hour for a 10-min period between 07h00 and 19h00. After each 10-min period the net was lowered and the flies trapped within were collected by means of a modified, portable vacuum cleaner and immediately placed inside a portable freezer for later identification and counting at the Blackfly Field Station in Upington.

During each 10 min trapping period the average air temperature and R.H. was recorded with a HANNA Instruments Thermo-Hygrometer (temperature accuracy = ± 0.4 °C at 25 °C; R.H. accuracy = ± 2 % at 25 °C). Average wind speed was recorded with an Extech Thermo-Anemometer (accuracy = ± 3 %) at a height of 1.2 m, and average light intensity was recorded with an Extech Light ProbeMeter (accuracy = ± 3 %). Wind direction, in relation to the Orange River, was also recorded during each sample period.

6.2.2 Statistical analyses

The average temperature, R.H., light intensity and wind speed were calculated for various times of the day over the entire study period. These values were then plotted against the average number of blackflies caught at various times of the day. Furthermore, correlations were calculated (using MS Excel) between blackfly numbers and temperature, R.H., light intensity and wind speed from the data collected over the entire study period. The tolerance range of *S. chutteri* to the above-mentioned variables was considered to be the minima and maxima for each variable where activity was recorded. As an additional test hourly blackfly numbers were plotted against temperature, R.H., wind speed and light intensity and linear and polynomial regressions were fitted to the data sets using MS Excel.

6.3 Results

The total number of flies caught per day, as well as the maximum, minimum and average hourly catches on each of the sampling days are shown in Table 6.1. Flies were only collected during seven of the 14 trials. The maximum number of flies caught in one trapping period was 86 (on 30/11/2000). The absence of flies during certain days can be attributed to the success of the blackfly control programme or unfavourable environmental conditions. All trial dates, on which a zero count was recorded, were excluded from the statistical analyses.

The average number of flies caught and the average temperature, R.H., wind speed and light intensity at various times of the day over the study period are shown in Fig. 6.1. It shows that blackfly activity was on average low from 07h00 to 08h00, but that it reached a peak from 09h00 to 10h00. This peak in activity corresponds with the period when temperature was low, although not at its lowest point. Correspondingly R.H. was high, although not at its highest point. These high numbers furthermore corresponds with a sharp increase in light intensity usually experienced at this time of day, while wind speed tended to be rather low. This suggests that these climatological factors should, to some degree, affect blackfly activity.

Table 6.1. Trial dates, total number of flies caught per day, maximum, minimum and average hourly catches recorded during the climatological trials.

Trial date	Total number of flies caught	Max. hourly catch	Min. hourly catch	Avg. hourly catch
5/10/1999	0	-	-	-
4/4/2000	214	54	0	17.8
7/8/2000	0	-	-	-
29/8/2000	15	4	0	1.3
30/11/2000	296	86	0	24.7
19/12/2000	213	46	0	17.8
6/3/2001	0	-	-	-
13/3/2001	0	-	-	-
24/3/2001	0	-	–	-
11/5/2001	187	48	0	15.6
17/5/2001	292	57	0	24.3
27/6/2001	0	-	-	-
8/8/2001	0	-	-	-
21/8/2001	0	-	-	-

Indeed, the correlation analyses show that light intensity was positively (and strongest) correlated to adult activity (r^2 = 0.2702). R.H. and temperature were also positively correlated with activity, although the relationship was not as strong as for light intensity (r^2 = 0.1213 and 0.0714 respectively). Only wind speed showed a negative correlation with adult activity (r^2 = -0.0695). Therefore, it can be hypothesized that blackfly activity will increase with an increase in light intensity, temperature and relative humidity, but that it will decrease with an increase in wind speed. To test this, adult numbers were plotted against temperature, R.H., wind speed and light intensity and both linear and polynomial regressions were fitted to the data (Fig. 6.2). The linear regression between adult numbers and temperature confirms that activity increased as temperature increased, but the polynomial regression gave a better fit. Therefore, activity will increase as temperature increases, but will start to decrease when temperature reaches a certain point. The optimum temperature for *S. chutteri* activity therefore lies between 24 – 32 °C (Fig. 6.3), although the temperature range in which activity was recorded was 15.3 – 41.0 °C (Table 6.2).

The linear regression fitted to R.H. and adult activity confirms a positive relationship between these two variables. However, as with temperature, the polynomial regression gave a better fit than the linear regression (Fig. 6.2). Therefore, blackfly activity increases as R.H. increases, but only up to a certain point, where activity starts to decrease. The optimum R.H. range for *S. chutteri* therefore lies between 35 and 50 %. Activity was, however, recorded between 4.5 and 53.5 % (Table 6.2), which can be considered their tolerance levels. However, R.H. only exceeded 53.5 % during two observation periods during the entire study. This was on a day when activity was very low, and therefore it is possible that *S. chutteri* will be active at higher humidities.

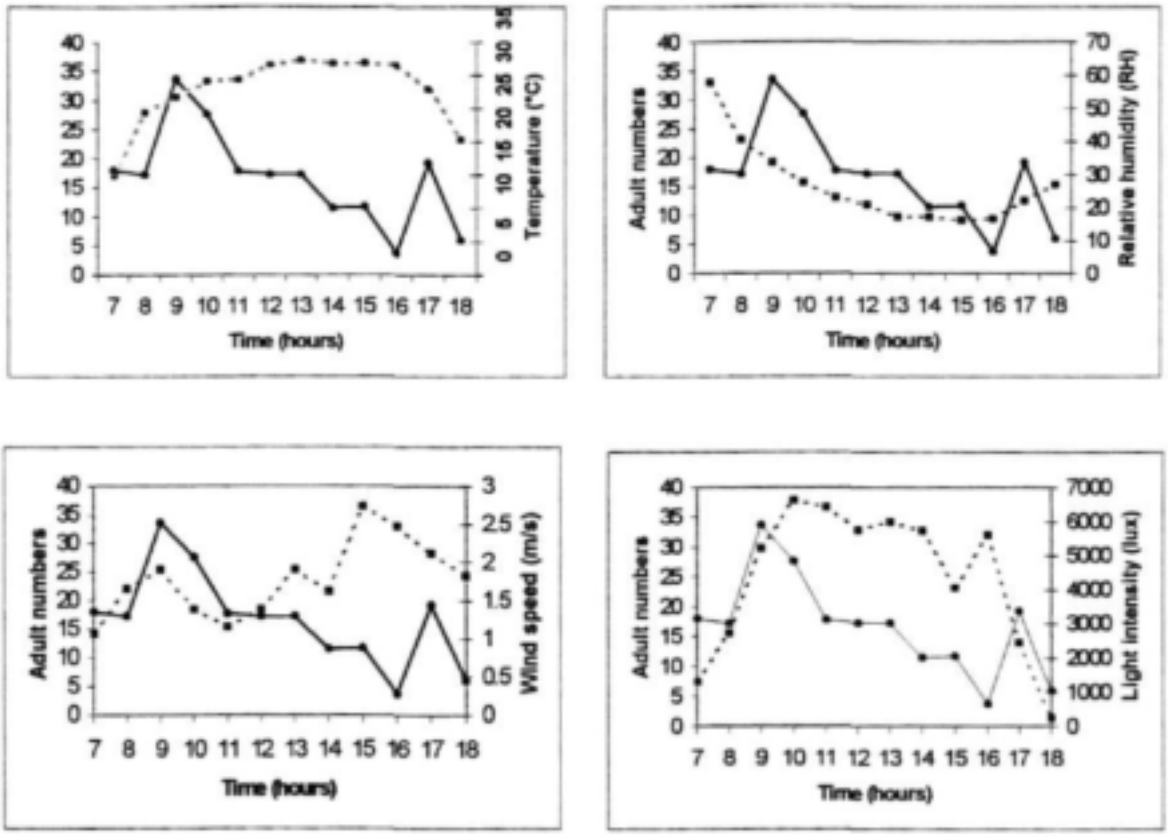


Fig. 6.1. The average number of blackflies (solid line) and average temperature , R.H. , wind speed and light intensity (broken lines) recorded at different times of the day throughout the study period.

The linear regression between wind speed and adult numbers were, as predicted by the correlation analyses, negative, and also gave a better fit than the polynomial regression (Fig. 6.2). Therefore, the activity of *S. chutteri* will decrease as wind speed increase, and in this study, no activity was recorded at wind speeds higher than 4.1 m/s (Table 6.2) Therefore, *S. chutteri* activity will be restricted to periods when wind speed is between 0 and 4.1 m/s.

The correlation analyses showed that adult activity is most closely related to light intensity and that activity increases as light intensity increases. This is confirmed by the linear and polynomial regressions that showed remarkably similar results. Therefore, it is confirmed that activity increases as light intensity increases. In this study activity was recorded between 30 and 9600 lux (Table 6.2), which should be considered their tolerance levels.

Table 6.2. The tolerance levels of *S. chutteri* to various climatological factors as recorded during the present study.

Climatological factor	**Minimum**	**Maximum**
Temperature (°C)	15.3	41.0
Wind speed (m/s)	0	4.1
Light intensity (lux)	30	9600
Relative humidity (%)	4.5	53.5

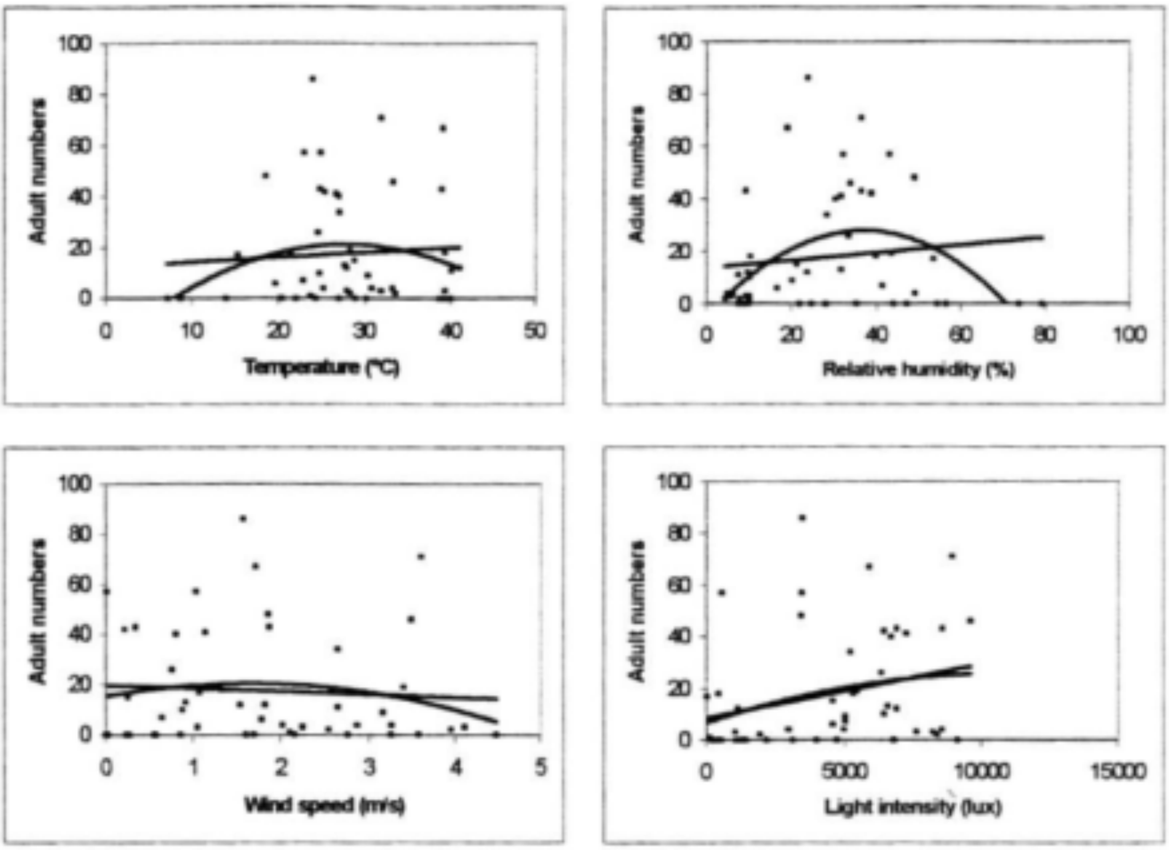


Fig. 6.2. Linear and second-degree polynomial regressions fitted to four climatological conditions and the activity of *S. chutteri* females.

1. Polynomial regression = $0.0537x^2 + 2.9848x - 20.296$, $r^2 < 0.05$
   Linear regression = $0.1921x + 12.264$, $r^2 < 0.05$
2. Polynomial regression: $r^2 = 0.211$
   Linear regression: $r^2 = 0.0147$
3. Polynomial regression: $r^2 = 0.0209$
   Linear regression: $r^2 = 0.0048$;
4. Polynomial regression = $-2E - 07x^2 + 0.0035x\ 6.7024$, $r^2 < 0.05$
   Linear regression = $0.0021x + 8.4642$, $r^2 < 0.05$

6.4 Discussion

In this study *S. chutteri* was active in a wide range of climatological conditions, including light intensities of 30 – 9600 lux, temperatures of 15.5 – 41.0 °C, wind speeds of 0 – 4.1 m/s and relative humidities of 4.5 – 53.5 %. Statistical analyses showed that light intensity had the largest affect on adult activity. This supports the hypothesis of Sutcliffe (1986), which states that light intensity is the factor that has the largest effect on adult activity and the effect of temperature, R.H. and wind speed is secondary. Light intensity seemed to be particularly important in the initiation of flight as no activity was recorded below 30 lux, even when conditions such as temperature and wind speed were favourable. Further proof for this hypothesis is provided by Begemann (1986) who reported that *S. chutteri* is not active during night. The threshold recorded here for *S. chutteri* is the same as that reported for *S. mettalicum* (Usova, 1961), slightly higher than that of *S. damnosum* (20 lux) (Steenkamp, 1972) and slightly less than that of *S. luggeri* (50 lux) (Fredeen and Mason, 1991).

S. chutteri activity increased as light intensity increased. This is consistent with the findings of Usova (1961) which showed that blackfly activity was highest between 500 and 11 000 lux. However, the results of the present study differ from the findings of Steenkamp (1972) in that he found the activity of *S. damnosum* decreased when light intensity was higher than 6200 lux.

Temperature was the second most important controlling factor of *S. chutteri* activity. This is in accordance with studies done by Steenkamp (1972) and Fredeen and Mason (1991) on other blackfly species. Adult *S. chutteri* activity was not recorded below 15.5 °C in this study, and the optimum temperature range was found to be between 24 and 32 °C. In his review on blackfly activity, Crosskey (1990) indicated that at temperatures between 8 and 10 °C flies are in complete torpor or too lethargic to fly, and that flight is usually only initiated at temperatures between 12 and 15 °C. Furthermore, Crosskey (1990) states that the optimum temperature range for flight in most species from the temperate regions is 15 – 25 °C, and for tropical species it is between 30 and 40 °C. Therefore, the optimum temperature range for *S. chutteri* is above that expected for temperate species and more in agreement with tropical species. However, a previous study on the effect of temperature on the activity of blackflies along the Vaal River, showed that adults were only active between 12 and 33 °C and a temperature range of 23 – 30 °C was considered the optimum (Steenkamp, 1972). This is consistent with the results reported here. Therefore, it seems that South African species, such as *S. damnosum* and *S. chutteri*, that have to survive in desert and semi-desert areas, are adapted for survival under higher temperature than those from other temperature areas. This hypothesis is strengthened by the findings of Palmer (1997) who previously noted that blackfly outbreaks along the Orange River are most likely to occur when the maximum air temperatures are between 20 and 35 °C.

The upper temperature limit at which activity was recorded for *S. chutteri* seems exceptionally high in comparison with other species. For example, the upper range at which activity was recorded for *S. damnosum* was 33 °C (Steenkamp, 1972) and 30.2 °C for *S. luggeri* (Fredeen and Mason, 1991) and, indeed, Palmer (1997) previously stated *S. chutteri* activity is generally low on hot days. However, Palmer (1997) also recorded that during days, when the maximum air temperature exceeded 38 °C, blackflies were found actively seeking favourable microclimates. Therefore, it is possible that the activity of *S. chutteri* recorded in the present study at the high temperatures (i.e. > 38 °C) was the result of adults seeking shelter in the shade provided by the trap.

In the present study blackfly activity was never recorded when wind speed exceeded 4.1 m/s, despite other factors, for example temperature and light intensity, being favourable. These findings are similar to that reported for *S. luggeri* (maximum wind speed ± 4.2

m/s) (Fredeen and Mason, 1991), *S. arcticum* (Maximum wind speed ± 4.4 m/s) (Shipp et al, 1987) and *S. venustum/verecundum* (Maximum wind speed ± 3.4 m/s) (McCreadie et al, 1986) and *S. damnsosum* (Maximum wind speed ± 3.2 m/s) (Steenkamp (1972). According to Crosskey (1990) wind can inhibit flight, but its importance rather lies more in whether flight can be maintained once airborne, than in the initiation of flight. Therefore, *S. chutteri* will be active up to 4.2 m/s, where after the high wind speeds will make the initiation of flight activity difficult, and perhaps, even impossible.

The results of this study showed that *S. chutteri* activity peak at humidities between 35 and 50 %. Furthermore, activity was recorded at R.H. as low as 4.5 %. According to Crosskey (1990) blackfly activity is generally low when the R.H. is very low (i.e. < 30 %) or very high (> 90 %) and the optimum lies between 40 and 80 %. Therefore, it seems that the optimum range for *S. chutteri* lies slightly lower than that expected for other species. This may either be attributed to the absence of high humidities during this study, or it may be an adaptation of *S. chutteri* to the low R.H. usually experienced along the lower Orange River (See Chapter 2). Nonetheless, *S. chutteri* is definitely more tolerant to high temperatures than other blackfly species. This has to be considered an adaptation to the harsh climatological conditions experienced in its habitat.

In conclusion, outbreaks of *S. chutteri* along the lower Orange River should be expected when the light intensity is higher than 30 lux (i.e. daytime) and when wind speeds are lower than 4.2 m/s. Moreover, outbreaks are most likely to occur when air temperature is between 24 and 32 ^{o}C and R.H. is between 35 and 50 %. The latter conditions are most relevant during spring and autumn (See Chapter 2) which is in accordance with the findings of Palmer (1997).

7. THE IMPACT OF BLACKFLY POPULATION DENSITIES ON THE DEFENSIVE BEHAVIOUR OF SHEEP

7.1 Introduction

Literature on blood feeding in blackflies is nearly void of references on host defensive behaviour, although it is acknowledged as an important factor influencing the survival of mosquitoes. For example, it has been shown that the defensive behaviour of certain vertebrates can successfully prevent attracted mosquitoes from feeding. Therefore, host behaviour plays an important role in determining the blood sources (Edman and Kale, 1970) and feeding success of mosquitoes (Klowden and Lea, 1979), and ultimately plays an important role in the epidemiology of mosquito-borne disease (Edman and Kale, 1970; Edman et al, 1972).

Furthermore, it has been well demonstrated in the mosquito literature that the density of biting mosquitoes directly affects the intensity of host defensive activity (Edman et al, 1974). More specifically, it is known that high mosquito densities cause increased host activity and that this results in a reduction in feeding success (Reeves, 1971, Klowden and Lea, 1978). Indeed, Edman et al (1974) has shown that the defensive behaviour of hosts may at times overshadow the importance of factors such as abundance, size and attractiveness, which are all components affecting the feeding success of mosquitoes.

Along the lower Orange River sheep under severe blackfly attacks bundle together with their heads stuck underneath each other. During these periods the sheep don't feed or mate, and this results in a loss in weight gain and a reduction in lambing percentages (Palmer, 1997). Apart from this there is no scientific data on the defensive behaviour used by sheep against blackfly attacks. This study was initiated to better understand the effect of blackfly attacks on sheep. The aims of the study were to record the types of host defensive behaviours used by sheep and to correlate these with blackfly abundance.

7.2 Materials and methods

These trials were done at the same time as the climatological trials reported on in Chapter 6. During each trial 50 ewes and 1 ram were enclosed in an encampment measuring 30 by 100 m. On every hour adults were collected for a period of 10 minutes as described in Chapter 6. During this time the behaviour of 10 sheep, picked at random from the group, were recorded for a period of 1 minute each, using a Sony TRV46E Hi8 video recorder. The recorded footage was copied to VHS format cassettes at the Blackfly Field Station. These cassettes were replayed on a television set and the behaviour of each of the sheep was studied and correlated with adult blackfly numbers collected in the trap at corresponding times.

To determine whether or not blackflies were responsible for the defensive behaviours recorded, the average number of times each response was recorded at the hourly intervals were calculated, over the entire study period, for the days when a zero blackfly count was recorded. This data was compared with the average number of times each response was recorded, at hourly intervals, for the days when the blackfly count exceeded zero. An ANOVA was used to determine the variance between samples.

7.3 Results

Five distinct behavioural responses of sheep were recorded (Table 7.1). The number of times each response was recorded in a 1-minute period was plotted against the number of adult blackflies collected in that period for the entire study (Fig. 7). The results show that ear twitches were far more common than the other responses. Least squares linear regressions were fitted to the plots and the results are shown in Table 7.2. The regression plot shows a significant linear relationship between adult blackfly numbers and the number of ear twitches. No significant relationships were, however, found between adult numbers and the other four behavioural responses (Table 7.2).

Table 7.1. Five behavioural responses recorded for sheep as a result of blackfly attacks.

Response	**Description**
Ear twitches	*Most common response recorded. Either one, or both, the ears are twitched (or shaken) in a rapid fashion. Serves to deter blackfly attacks on the face.*
Tail twitches	*The tail is shaken in a rapid fashion. Serves to deter blackfly attacks around the udder and anus.*
Head shaking	*Head of sheep is shaken from side to side. Sheep seem to be severely irritated. Serves to deter blackfly attacks on the face.*
Skin rippling	*The skin of the sheep is rippled. Similar to an animal shaking of excess water. Serves to deter attacks on the body.*
Feet stamping	*One foot at a time is stamped down on the ground. Sign of severe irritation. Serves to deter attacks on the ventral side of the sheep.*

To determine the reasons for the lack of relationships found, ANOVAS were calculated for each dataset. No significant variance was found within the samples obtained for the ear twitches ($p = 0.50$). However, significant differences were found within the samples of the other four behavioural responses ($p < 0.05$ in all four cases). These large variations within samples suggest that, except for ear twitches, individual sheep differ in their response to blackflies.

Table 7.2. Least squares linear regression analyses done on five behavioural responses of sheep on the number of blackflies trapped.

Behaviour	Slope ± SE	Intercept ± SE	r^2	p	F	n
Ear twitches	0.18 ± 0.07	13.59 ± 2.17	0.17	< 0.05	6.5	128
Tail twitches			0.02	0.48	0.51	128
Head shaking			0.04	0.44	0.78	128
Skin rippling			0.001	0.36	0.34	128
Feet stamping			0.003	0.80	0.069	128

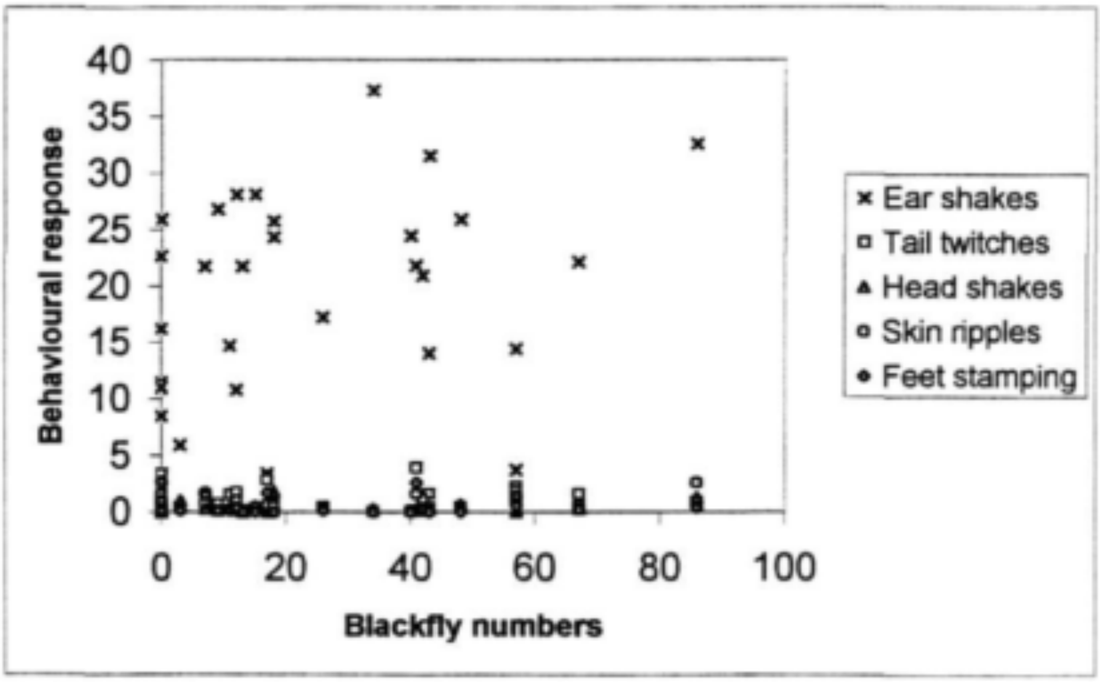


Fig. 7. The behavioural responses of sheep to the number of blackflies trapped.

The ANOVA showed that the average number of times sheep shake their ears are significantly higher when blackflies are present ($p < 0.001$). This is also true for feet stamping ($p < 0.001$), tail twitches ($p < 0.05$) and skin rippling ($p < 0.05$). The relationship between head shakes was, however, not significantly different ($p = 0.88$).

7.4 Discussion

In this study five distinct responses were recorded on sheep under attack from blackflies. Each of these had a specific purpose and served to deter attacks on various parts of the body. The most common response recorded was shaking of the ears. This served to prevent attacks on the ears and face area, which are acknowledged as being preferred areas of attack (Palmer, 1997). Tail twitches were used by the sheep to prevent attacks around the anus and udder areas. These are also known target areas (Crosskey, 1990). However, this response was not as common as ear shaking. The other three responses, namely head shaking, skin rippling and foot stamping, seemed to be coupled with severe irritation to the sheep. Head shaking deters attacks around the head, foot stamping protects the less protected areas on the ventral part of the body and skin rippling protects the sheep from attacks on the dorsal part of the body. However, statistical analyses showed that head shakes is not a response to blackfly attacks. It can therefore be argued that this response is the result of other pests, such as noseflies. However, further studies are needed to satisfactorily prove this.

No bundling behaviour was recorded during this study, although it is regarded as a common response to blackfly attacks under severe outbreaks (Palmer, 1997). During the present study more than 50 blackflies were frequently trapped in a 10-min period. During such periods the observers were severely troubled by blackflies, but the sheep only responded by increasing their ear twitching frequency. Therefore, it seems that sheep will only bundle when they are under severe attack from blackflies, or when they are subjected to periods of extended attacks.

The only significant correlation between a behavioural response and adult numbers was recorded for the number of ear twitches. This, first of all, suggests that the ears are the preferred feeding area of simuliids. Secondly, the lack of correlation found between blackfly numbers and the other responses either suggest that individual sheep differ in their response to blood-sucking blackflies, or these responses may be in reaction to blackflies attacking sensitive parts of the body, e.g. the udders and anus. Support for the latter hypothesis is given by the fact that all four these behavioural responses were recorded when blackfly numbers were also very low. However, differences in defensive behaviour are not only commonly recorded between species (Edman et al, 1971; Edman et al, 1974), but it has also been shown that individual animals also differ in their response to both mosquitoes (Mayer and James, 1969; Smith et al, 1962) and blackflies (Crosskey, 1990). Although these differences are often attributed to variations in breath, smell and other substances on the skin (Thompson, 1976a, b), Edman and Kale (1971) showed that mosquitoes rejected by one host will find a more submissive host which will, therefore, be prone to more attacks. In this study it was clearly demonstrated that different sheep exhibit different responses to blackflies (except for ear shaking). Therefore, the animals with lesser defense mechanisms are likely to be prone to the worst attacks and will show more mutilation than the others. Although this was not directly measured in this study, observations confirm that some sheep have worse bite wounds than other sheep. These wounds could not be attributed to age or body mass (unpublished data).

The differences in the response of sheep to blackfly attacks are also important in disease transmission. For example, Klowden and Lea (1979) showed that the defensive behaviour of rabbits cause mosquitoes to feed on more than one host. This increases the chances of acquiring parasites from an infected host, as well as disseminating infections to other hosts. If this happens for blackflies, when feeding on sheep, it may increase their vector potential.

In conclusion, the primary method of protection used by sheep is to ward off feeding blackflies through ear shaking, while the rest of the body is protected by secondary defensive behaviour. Moreover, within a population different sheep utilize different methods of protection, which enhances the probability of disease transmission. The results of this study should be seen as the basis for future studies on the defensive behaviour of sheep and the feeding success of blackflies. Furthermore, the results showed the significant correlation between ear twitches and adult blackfly numbers and therefore it is believed that this defensive behaviour can be used to indicate the level of adult annoyance and may therefore be used, for example, to test the efficacy of various repellants.

8. GENERAL DISCUSSION

8.1 Implications of the present study

This study has shown that the body size, mass and lipid content of *S. chutteri* are inversely related to larval developmental (or water) temperature. As a result of these inverse relationships, seasonal variation occurred in simuliid size, mass and lipid content. The largest specimens with the largest lipid reserves developed during winter at the coldest water temperatures, and the smallest individuals with the least reserves developed during summer, at the highest water temperatures. Moreover, specimens developing further downstream, at warmer temperatures, were smaller and carried less metabolic reserves than those that developed further upstream. These patterns are typical of those expected for small ectotherms (Atkinson, 1994), and similar patterns have been well documented in the Simuliidae literature (Crosskey, 1990). Although the studies on the glycogen content of *S. chutteri* showed no relationship between this variable and developmental temperature, some variation was found in the samples used in the longevity trials. This suggest that experimental errors occurred in either the analyses of the seasonal glycogen content or those used in the longevity trials. Nonetheless, larger specimens have a higher glycogen mass than smaller specimens and it follows that that the glycogen content of *S. chutteri* does indeed vary seasonally.

Major seasonal variations were also found in the longevity of *S. chutteri* adults that were kept under a range of temperature and relative humidity combinations. Despite these variations, longevity in all the populations decreased as temperature increased and humidity decreased.

The importance of rainfall in the survival of adult blackflies was shown, as more carbohydrate sources were available after periods of rainfall. This meant that adults did not only have a greater variety to choose from, but flowers were also more abundant. Furthermore, plant species within the drainage lines are the ideal nectar sources and shelters for adult blackflies. It is also argued that the widespread *Acacia mellifera* contributes significantly to the survival of adult blackflies. It is concluded that carbohydrate scarcity is not a limiting factor to the survival of adult blackflies along the lower Orange River.

Lastly, the annoyance level of *S. chutteri* along the lower Orange River should be highest when the light intensity is above 30 lux (i.e. daytime), when wind speeds are below 4.2 m/s, when air temperature is between 24 and 32 °C, and when R.H. is between 35 and 50 %. The latter conditions are most relevant during spring and autumn.

It is argued that these scientifically evaluated variables are all important factors influencing the population dynamics of *S. chutteri*. They can also be used, to some extent, to explain the typical seasonal variation found in the annoyance levels exhibited by adult blackflies along the lower Orange River. Therefore, outbreaks can be predicted with more accuracy and can be adjusted accordingly (See Chapter 9).

8.2 Blackfly seasonality – physiological and ecological perspectives

8.2.1 Winter population

The winter population discussed here refers to the flies that were used in the longevity trials and which eclosed on 10 July 2000. This population developed at 11 °C and would therefore have taken approximately 5 to 6 weeks to develop to the adult stage (Palmer, 1997). The winter population had the highest mean body size, mass, lipid and glycogen content and this was strongly associated with the low developmental temperature.

During mid-winter (June and July) adult blackfly annoyance is usually low along the lower Orange River (Jordaan and Van Ark, 1990) and circumstantial evidence suggests that this is because *S. chutteri* do not pupate at temperatures below 10 °C and therefore no adults emerge at that time (Palmer, 1997). However, during the present study water temperature exceeded 10 °C in July at Gifkloof (See Chapter 3) and therefore vast numbers of pupae were recorded in the Orange River at that time (data not shown). Despite the high pupal abundance, adult annoyance remained low during July and early August 2000 (E. Myburgh, pers. obs.).

I propose the following hypothesis for the low adult activity usually experienced during the winter months. An increase in ectotherm body size is generally associated with increased fecundity (Chutter, 1970; Colbo, 1982, Merritt et al,1982; Ross and Merritt, 1987; Akoh et al, 1992; Baba, 1992; Hadi and Takaoka, 1995; Baba, 1992; Takken et al, 1998). Although fecundity was not directly measured in the present study, previous studies on *S. chutteri* showed that the first gonotrophic cycle (i.e. the winter population) develops its eggs autogenously (Begemann, 1986). Therefore, this population should, as predicted in the relevant literature, have the highest potential fecundity of all the populations. Autogeny not only increases potential fecundity, but also permits the winter population to exist beyond the ecological borders of others, as they do not have to search for a blood meal and thus be subjected to unfavourable environmental conditions and predation (Crosskey, 1990). This will therefore tend to increase their longevity in nature.

Just as size is an indicator of potential fecundity in small ectotherms, an increase in the lipid and glycogen of individuals generally increases their resistance to stresses such as desiccation, starvation and heat, and this ultimately leads to increased longevity (Service et al, 1985; 1988; Service, 1987; Mogi et al, 1996). However, in the present study these correlations were not found. The winter population showed the lowest survival of all the populations under almost all the conditions tested. It is argued in this report, that despite the obvious advantages of autogeny, it also has a negative impact by diverting energy and other resources away from important fitness traits such as stress resistance. This can potentially lead to decreased longevity in the winter *S. chutteri* population in comparison to the others (See Chapter 4) , were they to have been subjected to summer climatological conditions. Therefore, it seems that there may be a life history trade-off between fecundity and longevity. This is not unusual and has been found in several other invertebrates (Gillies, 1964; Akoh et al, 1992; Graves et al, 1992).

However, this does not imply that the winter population does not contain the necessary mechanisms required for long periods of survival. Owing to their autogenous nature winter females do not require a blood meal for ovarian development (Crosskey, 1990), and therefore do not need to disperse away from their breeding sites. In the vegetation along the Orange River temperatures are generally lower and the relative humdity higher than in the adjacent vegetation (Myburgh et al, 2002). Thus, it is likely that blackflies will rather take shelter in shrubs and trees associated with the drainage lines of the Orange River and its tributaries. Moreover, the climatological conditions experienced at this time of the year (See Chapter 2) are the most favourable for adult blackfly survival (Begemann, 1986), although the low temperatures will severely limit activity. Thus, by not being exposed to heat, desiccation and starvation this population can potentially survive considerable periods of time as predicted by the longevity plots presented in Chapter 4. Furthermore, plant species such as *Sisyndite spartea* and *Tamarix usneoides*, used as food sources by *S. chutteri*, flower at this time of the year (Chapter 5) and therefore nectar will be readily available and this will further enhance their survival.

It therefore seems that although adults are likely to be present in large numbers during winter, and have the ability to survive for long periods, they are not regarded as troublesome as they do not readily feed on animal hosts. The importance of the winter population thus lies, not in its high annoyance levels, but rather in its ability to successfully deposit vast numbers of eggs in the Orange River - which leads to potentially major population outbreaks during spring.

8.2.2. Spring population

The spring population discussed here refers to the adults that emerged on 10 September 2000, and which were used in the longevity trials. They developed at 19 °C with an approximate developmenl period of 3 to 4 weeks (Palmer, 1997). Their body size, mass, lipid and glycogen contents were lower than the winter population, but higher than the summer and autumn populations.

The largest blackfly outbreaks along the Orange River usually occur during spring each year (Jordaan and Van Ark, 1990; Palmer, 1997). The present study showed that many factors are responsible for this increase in biting annoyance. Firstly, this population exhibited the highest desiccation and starvation tolerance of all the populations tested. This high tolerance to stresses suggest that they are physiologically best equipped for leaving their breeding sites, successfully feeding and returning to the Orange River, the only available breeding site in the area, to oviposit.

Moreover, previous studies on blood-feeding Diptera, such as Culicidae, showed that an increase in body size is generally associated with increased dispersal ability (Thomas, 1993). Larger specimens also more readily engage in host-seeking behaviour and feed more successfully (Klowden et al, 1988; Nasci, 1991) by manifesting higher host attack rates than smaller specimens. Therefore, larger individuals also have higher vector potentials than smaller ones (Xue and Barnard, 1996). As the spring population had the largest body size of all the blood-feeding populations, it is likely that their high annoyance levels, in comparison to the other populations, can be attributed to their ability to disperse relatively far, which will potentially bring them into contact with more hosts. Furthermore, if they do more readily engage in host-seeking behaviour and feed more successfully than the other populations, an increase in biting frequency can be expected. (Nasci, 1991).

Also, blackfly populations tend to overlap at this time of the year which also leads to an increase in population densities (De Moor, 1982b). In addition to the these factors, larval numbers are generally very high during spring (Palmer, 1997), which means that vast numbers of adults will emerge from the Orange River at that time. During spring, environmental conditions are not as harsh as those experienced over the summer months (See Chapter 2) and activity is therefore likely to be at its maximum (Chapter 6). This, together with the fact that one of their favourite, abundant and widespread nectar sources, *Acacia mellifera* (Chapter 5) flowers at this time of the year, will further favour this population.

Therefore, a combination of physiological, ecological and climatological factors ensures that this population is capable of surviving for a considerable period and thus exhibits high annoyance levels.

8.2.3. Summer population

In comparison to the other populations, the individuals that developed during summer were relatively small and had low glycogen and lipid reserves. This is due to their rapid development (7 to 10 days) (Palmer, 1997) associated with the high water temperature (26 °C). In this discussion the summer population refers to adults that emerged from pupae on 10 January 2001.

It is hypothesized, although not yet satisfactorily proven, that blackfly numbers are generally low during the summer months because the summer climatological conditions severely limit adult survival (Palmer, 1997). Despite this, major outbreaks do occur periodically during the summer months.

The present study shows that the summer population had lower desiccation and starvation resistance than the spring and autumn populations, although this is the population that is most likely to be exposed to these conditions (See Chapter 4). Moreover, the longevity of this population under laboratory conditions was less than 24 h at temperatures exceeding 27 °C. This temperature is frequently exceeded during the summer months (See Chapter 2) and therefore it appears that the longevity of the summer population will be severely limited. As this population also emerges with low metabolic reserves they would need to take a sugar meal as soon as possible after emergence to enhance their survival (Crosskey, 1990). This, in turn, exposes them to the harsh climatological conditions and should result in rapid mortality.

Therefore, the probability of a summer outbreak seems highly unlikely. However, as summer outbreaks do occur from time to time, it is clear that some other factors may be involved which counter the negative effect of harsh climatological conditions. For example, certain populations may be favoured by mild climatological conditions, high rainfall may lead to an abundance of nectar sources, or consistently high water flows may give rise to abnormally high breeding populations in the river.

8.2.4. Autumn population

The autumn population discussed here refers to the adults that were hatched on 10 April 2001 and which were used in the longevity trials. They developed at 22 °C with an approximate development time of 2 to 3 weeks (Palmer, 1997). Their body size, mass, lipid and glycogen contents were lower than the winter and spring populations, but higher than the summer population.

Although population outbreaks frequently occur over the autumn period along the Orange River, the problem is seldom as severe as that experienced in the spring. This can, in part, be attributed to individuals that develop during autumn being slightly smaller and having less lipid and glycogen reserves than the spring population. Therefore, the arguments discussed under the spring population are also relevant here, although the smaller size and less reserves result in lower annoyance levels.

9. RECOMMENDATIONS

9.1 Current recommendations for blackfly control

It is currently recommended that blackfly control operations along the lower Orange River only be implemented from July – October and from March – April (Palmer, 1997), owing to the increase in adult annoyance usually experienced during these periods.

9.2 New recommendations for blackfly control

The recommendations discussed under section 9.1 have been questioned in recent years because periodic outbreaks have occurred during mid-summer. The results of the present study, based on the physiological and ecological requirements of *S. chutteri*, are used to make the following recommendations for the improvement of the control programme:

- The results of the present longevity and physiological study showed that the probability of a summer outbreak is indeed very low, as longevity will be severely limited over the summer months by the extreme temperatures experienced at this time. However, as outbreaks do occur over the summer months, either *S. chutteri* is capable of using other mechanisms to overcome these harsh conditions, or outbreaks are restricted to cooler years of higher rainfall, or consistently high water flows result in abnormally high blackfly breeding populations. Thus, it is recommended that larvicide applications be considered during the summer months, but only after and when:

1. An objective monitoring programme has been restored. This will serve to quantify the nature of the summer outbreaks and will also assist in predicting longevity under field conditions;
2. Larval numbers warrant control (i.e. exceeding 100 000m^2);
3. Periods of favourable environmental conditions arise (i.e. low temperature and high RH) that will favour adult survival;
4. Studies have been conducted on the mechanisms utilized by *S. chutteri* to overcome harsh environmental conditions;
5. The effect of high water flow on larval and pupal populations is understood.

- It is recommended that one dedicated and qualified person be appointed to take responsibility for all monitoring aspects, as objective and accurate monitoring is crucial to the success of the control programme.

- If the monitoring system can be improved a more flexible approach should be adapted where the results of the monitoring should be incorporated in the planning of control actions.

- This study showed the importance of the winter larval population in giving rise to major outbreaks of adults during the spring period. These adults were also shown to survive the longest. Taking all these factors into account it is clear that every effort should be made to effectively control the spring population before it emerges and lays eggs, especially as this population may be autogenous.

- It is recommended that the blackfly control operators be made aware of the limitations of high temperatures and low humidities on adult blackfly survival and that control actions be adjusted accordingly, i.e. that control actions be suspended when the longevity plots indicate blackfly longevity will severely be affected. This will hopefully serve to prevent unnecessary larvicide applications and therefore make the control programme more cost-efficient.

10. REFERENCES

Alverson, D.R., Noblet, R. 1976. Response of female black flies to selected metereological factors. *Environmental Entomology*, 5: 662 – 665.

Akoh, J.I., Aigbodian, F.I., Kumbak, D. 1992. Studies on the effect of larval diet, adult body weight, size of blood-meal and age on the fecundity of *Culex quinquefasciatus* (Diptera: Culicidae). *Insect Science and its Application*, 13: 177 – 181.

Anderson, J.R., Voskuil, G.H. 1963. A reduction in milk production caused by the feeding of blackflies (Diptera: Simuliidae) on dairy cattle in California, with notes on the feeding activity on other animals. *Mosquito News*, 23: 126 – 131.

Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms? *Advances in Ecological Research*, 25: 1 – 58.

Atkinson, D., Sibly, R.M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution*, 12: 235 – 239.

Baba, M. 1992. Oviposition habits of *Simulium kawamurae* (Diptera: Simuliidae), with reference to seasonal changes in body size and fecundity. *Journal of Medical Entomology*, 29: 603 – 610.

Barker, J.F., Barker, A. 1980. The relation between body size and resistance to desiccation in two species of *Zaprionus* (Drosophiliidae). *Ecological Entomology*, 5: 309 – 314.

Bates, M. 1947. The development and longevity of haemagogus mosquitoes under laboratory conditions. *Annals of the Entomological Society of America*, 7: 1 - 12.

Beck, S.D. 1983. Insect thermoperiodism. *Annual Review of Entomology*, 28: 91 – 108.

Begemann, G.J. 1980. Laboratory studies on the biology of *Simulium nigritarse* Coquillett and *Simulium adersi* Pomeroy (Diptera: Simuliidae). *Onderstepoort Journal of Veterinary Research*, 47: 203 – 211.

Begemann, G.J. 1986. *Die bionomie van sekere Suid-Afrikaanse Simuliidae-spesies*. M.Sc thesis. University of Pretoria.

Bernado, M.J., Cupp, E.W. 1986. Rearing black flies (Diptera:Simuliidae) in the laboratory: mass scale in vitro membrane feeding and its application to collection of saliva and parasitological and repellant studies. *Journal of Medical Entomology*, 23: 666 – 679.

Berrigan, D., Charnov, E.L. 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos*, 70: 474 – 478.

Bezuidenhout, H. 1996. The major vegetation communities of the Augrabies Falls National Park, Northern Cape. 1. The southern section. *Koedoe* 39(2): 7 – 24.

Brenner, R.J., Cupp, W. 1980. Preliminary observations on parity and nectar feeding in the black fly, *Simulium jenningsi*. *Mosquito News* 40(3): 390 – 393.

Bridges, V.E., McCluskey, B.J., Salman, M.D., Hurd, H.S., Dick, J. 1997. Review of the 1995 vesicular stomatitis outbreak in the western United States. *Veterinary Medicine Today*, 211: 556 – 560.

Briegel, H. 1990. Fecundity, metabolism and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *Journal of Medical Entomology*, 27: 839 – 850.

Brown, A.W.A. 1962. A new insecticide for the control of blackflies (Diptera: Simuliidae). *Bulletin of the World Health Organization*, 27: 511- 522.

Burgin, S.G., Hunter, F.F. 1997a. Nectar versus honeydew as sources of sugar for male and female black flies (Diptera: Simuliidae). *Journal of Medical Entomology*, 34: 605 – 608.

Burgin, S.G. , Hunter, F.F. 1997b. Evidence of honeydew feeding in black flies (Diptera: Simuliidae). *Canadian Entomologist* 34: 124 – 132.

Burgin, S.G., Hunter, F.F. 1997c. Sugar meal sources of female black flies (Diptera: Simuliidae): a four habit study. *Canadian Journal of Zoology* 75: 1066 – 1072.

Bursell, E. 1974. The measurement of size in tsetse flies (Glossina*)*. *Bulletin of Entomological Research*, 51: 33 – 37.

Car, M. 1983. The influence of water-level fluctuation on the drift of *Simulium chutteri* Lewis 1965 (Diptera: Nematocera) in the Orange River, South Africa. *Onderstepoort Journal of Veterinary Research*, 50: 173 – 177.

Car, M. 1984. Laboratory and field trials with two *Bacillus thuringiensis* var. *israelensis* products for *Simulium* (Diptera: Nematocera) control in a small polluted river in South Africa. *Onderstepoort Journal of Veterinary Research*, 51: 141-144.

Car, M., De Moor, F.C. 1984. The response of Vaal River drift and benthos to *Simulium* (Diptera: Nematocera) control using *Bacillus thuringiensis* var. *israelensis* (H-14). *Onderstepoort Journal of Veterinary Research*, 51: 155 - 160.

Chown, S.L., Gaston, K.J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews*, 74: 87 – 120.

Chutter, F.M. 1963. Hydrobiological studies on the Vaal River in the Vereeniging area. Part 1. Introduction, water chemistry and biological studies on the fauna of habitats other than muddy bottom sediments. *Hydrobiologica*, 21: 1 - 65.

Chutter, F.M. 1968. On the ecology of the fauna of stones in the current in a South African river supporting a very large *Simulium* population. *Journal of Applied Ecology*, 5: 531-561.

Chutter, F.M. 1970. A preliminary study of factors influencing the number of oocytes present in newly emerged blackflies (Diptera: Simuliidae) in Ontario. *Canadian Journal of Zoology*, 48: 1389 – 1400.

Chutter, FM., Palmer, R.W., Walmsley, J.J. 1996. *Orange River development project replanning study. Environmental overview of the Orange River*. Department of Water Affairs and Forestry Report No. PD000/00/5292.

Clark, A., Doane, W.W. 1983. Desiccation tolerance of the adispose mutant *of Drosophila melanogaster*. *Hereditas*, 99: 165 – 175.

Coetzee, A.N. 1982. *The population structure and dynamics of* Simulium *in the Great Fish River*. M.Sc. thesis. Rhodes University.

Colbo, M.H. 1982. Size and fecundity of adult Simuliidae (Diptera) as a function of stream habitat, year and parasitism. *Canadian Journal of Zoology*, 60: 2507 – 2513.

Colbo, M.H., Porter, G.N. 1979. Effects of food supply on the life history of Simuliidae (Diptera: Simuliidae). *Canadian Journal of Zoology*, 57: 301 – 306.

Colbo, M.H., Porter, G.N. 1981. The interaction of rearing temperature and food supply on the life history of two species of Simuliidae (Diptera). *Canadian Journal of Zoology*, 59: 158 – 163.

Collins, R.C., Merino, M.E., Cupp, E.W. 1981. Seasonal and diurnal patterns of man-biting activity of four species of Guatemalan black flies (Diptera: simuliidae). *American Journal of Tropical Medicine and Hygiene*, 30: 728 – 733.

Corbet, P.S. 1964. Autogeny and oviposition in Arctic mosquitoes. *Nature* 203: 668 – 678.

Crosskey, R.W. 1990. *The natural history of blackflies*. John Wiley and Sons: Chichester.

Crosskey, R.W. 1993. Blackflies (Simuliidae). In: *Medical Insects and Arachnids*. Eds. Lane, P., Crosskey, R.W. John Wiley and Sons: Chichester.

Cupp, E.W., Collins, R.C. 1979. The gonotrophic cycle in *Simulium ochraceum*. *American Journal of Tropical Medicine* 28: 422 – 426.

Cupp, E.W. 1981. Blackfly physiology. *In*: LAIRD, M. (Ed.). *Blackflies. The future for biological methods in integrated control*. London: Academic Press.

Da Lage, J-L., Capy, P., David, J-R. 1989. Starvation and desiccation tolerance in *Drosophila melanogaster* adults: effects of environmental temperature. *Journal of Insect Physiology*, 35: 453 – 457.

Dalmat, H.T. 1952. Longevity and further flight range studies on the blackflies (Diptera: Simuliidae), with the use of dye markers. *Annals of the Entomological Society of America*, 45: 23 – 27.

Davies, J.B. 1994. Sixty years of Ochocerciasis Vector Control: A chronological summary with comments on eradication, reinvasion, and insecticidal resistance. *Annual Review of Entomology*, 39: 23 – 45.

Davies, D.M., Peterson, B.V. 1956. Observations on the mating, feeding, ovarian development and oviposition of adult black flies (Simuliidae, Diptera). *Canadian Journal of Zoology*, 34: 615 – 655.

Davies, D.M., Peterson, B.V., Wood, D.M. 1962. The black flies (Diptera: Simuliidae) of Ontario. Part I. Adult identification and distributions of six new species. *Proceedings of the Entomological Society of Ontario,* 92: 70 – 154.

De Moor, F.C. 1982a. Determination of the number of instars and size variation in the larvae and pupae of *Simulium chutteri* Lewis 1965 (Diptera: Simuliidae) and some bionomical implications. *Canadian Journal of Zoology*, 60: 1374 – 1382.

De Moor, F.C. 1982b. *A community of Simulium species in the Vaal River near Warrenton.* Ph.D. thesis. Vol 1. University of the Witwatersrand.

De Moor, F.C. 1986. Blackfly problems in and along the Vaal River. In: *The Vaal River ecosystem: Status and problems. Proceedings of a joint symposium convened by the Foundation for Research and Development and the Vaal River Catchment Association*, 159 - 173.

De Moor, F.C., Car, M. 1986. A field evaluation of *Bacillus thuringiensis* var. *israelensis* as a biological control agent for *Simulium chutteri* (Diptera: Nematocera) in the middle Orange River. *Onderstepoort Journal of Veterinary Research,* 53: 43 – 50.

De Villiers, P.C. 1987. *Simulium* dermatitis in man – clinical and biological features in South Africa. A case report. *South African Medical Journal*, 71: 523 – 525.

Dwarakanath, S.K., Berlin, O.G., Subramanian, M., Selvaraj Pandian, R. 1974. Reactions of *Culex fatigans* Weidemann (Diptera Culicidae) to the relative humidity in the air. *Monitore zoologica italiano,* 8: 205 – 213.

Edman, J.D., Kale, H.W. 1971. Host behavior: Its influence on the feeding success of mosquitoes. *Annals of the Entomological Society of America*, 64: 513 – 516.

Edman, J.D., Simmons, K.R. 1985. Rearing and colonization of black flies (Diptera: Simuliidae). *Journal of Medical Entomology*, 22: 1 – 17.

Edman, J.D., Webber, L.A., Kale, H.W. 1972. Effect of mosquito density on the interrelationship of host behavior and mosquito feeding success. *The American Journal of Tropical Medicine and Hygiene*, 21: 487 – 491.

Edman, J.D., Webber, L.A., Schmid, A.A. 1974. Effect of host defenses on the feeding pattern of *Culex nigriphalus* when offered a choice of blood sources. The Journal of Parasitology, 60: 874 – 883.

Edwardes, M., Palmer, R.W. 1994. *The extent of the blackfly problem in South Africa.* Unpublished report to the National Department of Agriculture.

Ernsting, G., Isaaks, J.A. 1997. Effects of temperature and season on egg size, hatching size and adult size in *Notiophilus biguttatus*. *Ecological Entomology*, 22: 32 – 40.

Everson, C.S. 1999. *Evaporation from the Orange River: Quantifying open water resources.* Water Research Commission report no. 683/1/99.

Fabricius, C., Van den Berg, P. 1993. The phenology of some locally common browse species in an arid savanna. *Bontebok* 8: 31 – 32.

Fredeen, F.J.H., Mason, P.G. 1991. Metereological factors influencing host-seeking activity of female *Simulium luggeri* (Diptera: Simuliidae). *Journal of Medical Entomology*, 28: 831 – 840.

French, V., Feast, M., Partridge, L. 1998. Body size and cell size in *Drosophila*: the developmental response to temperature. *Journal of Insect Physiology*, 44: 1081 – 1089.

Fuller, C. 1899. A new poultry pest. *Agricultural Journal of the Cape of Good Hope*, 14: 29 – 35.

Fuller, C. 1913. Fly plagues, an unusual outbreak of *Stomoxys calcitrans* following floods. *Agricultural Journal of the Union of South Africa*, 922 – 925.

Gibbs, A.G., Chippendale, A.K., Rose, M.R. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *The Journal of Experimental Biology*, 200: 1821 – 1832.

Gibson, G., Torr, S.J. 1999. Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology*, 13: 2 – 23.

Gillies, M.T. 1964. The study of longevity in biting insects. *International Review of General and Experimental Zoology*, 1: 47 – 76.

Graves, J.L., Toolson, E.C., Jeong, C., Vue, L.N., Rose, M.R. 1992. Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiological Zoology*, 65: 268 – 286.

Hadi, U. K., Takaoka, H. 1995. Effects of constant temperature on oviposition and immature development of *Simulium bidentatum* (Diptera: Simuliidae), a vector of bovine Onchocerca (Nematoda: Onchocercidae) in central Kyushu, Japan. *Journal of Medical Entomology*, 32: 801 – 806.

Hadley, N.F. 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armata*: seasonal and acclamatory effects of composition. *Insect Biochemistry*, 7: 277 – 283.

Hancock, R.G., Foster, W.A. 1997. Larval and adult nutrition effects on blood/nectar choice of *Culex nigripalpus* mosquitoes. *Medical and Veterinary Entomology*, 11: 112 – 122.

Hauer, F.R., Benke, A.C. 1987. Influence of temperature and river hydrograph on black fly growth rates in a subtropical blackwater river. *Journal of the North American Benthological Society*, 6: 251 – 262.

Hochachaka, P.W., Somero, G.N. 1985. *Biochemical adaptation*. Princeton University Press: New York.

Hocking, B. 1953. The intrinsic range and speed of flight of insects. *Transactions of the Royal Entomological Society of London*, 99: 475 – 496.

Hoffman, A.A. 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *Journal of Insect Physiology*, 36: 885 – 891.

Hoffman, M.T. 1996. Orange River Nama Karroo. In: *Vegetation of South Africa, Lesotho and Swaziland. Eds.* Low, A.B., Rebelo, A.G. Department of Environmental Affairs and Forestry: Pretoria.

Hoffman, M.T. 1989. A preliminary investigation of the phenology of subtropical thicket and karroid shrubland in the lower Sundays River Valley, SE Cape. *South African Journal of Botany* 55(6): 586 – 597.

Hoffman, M.T. 1996. Orange River Nama Karoo. *In:* LOW, A.B. & A.G. REBELO (eds.). *Vegetation of South Africa, Lesotho and Swaziland.* Pretoria: Department of Environmental Affairs and Tourism.

Hoffman, A.A., Parsons, P.A. 1989a. An integrated approach to environmental stress tolerance and life history variation: desiccation tolerance *in Drosophila. Biological Journal of the Linnaean Society,* 37: 117 – 136.

Hoffman, A.A., Parsons, P.A. 1989b. Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics*, 122: 837 – 845.

Hougard, J-M., Yaméogo, L., Sékétéli, A., Boatin, B., Dadzie, K.Y. 1997. Twenty-two years of blackfly control in the Onchocerciasis control programme in West Africa. *Parasitology Today*, 13: 425 – 431.

Howell, C.J., Holmes, G.W. 1969. The control of Simuliidae in the Vaalharts Irrigation Complex. *Journal of the South African Veterinary Association*, 40: 59 - 67.

Howell, C.J., Begemann, G.J., Muir, R.W., Louw, P. 1981. The control of Simuliidae (Diptera: Nematocera) in South African rivers by modification of the water flow volume. *Onderstepoort Journal of Veterinary Research,* 48: 47- 49.

Huey, R.B., Crill, W.D., Kingsolver, J.G., Weber, K.E. 1992. A method for rapid measurement of heat or cold resistance of small insects. *Functional Ecology*, 6: 489 – 494.

Hunter, D.M. 1977. Sugar-feeding in some Queensland black flies. *Journal of Medical Entomology*, 14: 229 – 232.

Jordaan, L.C., Van Ark, H. 1990. A survey of annoyance of livestock by *Simulium chutteri* Lewis along the Orange River, South Africa (Diptera: Simuliidae). *Onderstepoort Journal of Veterinary Research,* 57: 189 – 195.

Jordan, S., Hubbard, S.F. 1991. Influence of humidity and temperature on the diel periodicity of oviposition of *Toxorhynchites moctezuma* (Diptera: Simuliidae) in the field. *Entomological Society of America*, 28: 241 – 245.

Joshi, D.S. 1995. Effect of fluctuating and constant temperatures on development, adult longevity and fecundity in the mosquito *Aedes krombeini*. *Journal of Thermal Biology*, 21: 151 – 154.

Junge-Berberović, P.R. 1996. Effect of thermal environment on life histories of free living *Drosophila melanogaster* and *D.subobscura*. *Oecologia*, 108: 262 – 272.

Kari, J.S., Huey, R.B. 2000. Size and seasonal temperature in free-ranging *Drosophila subobscura*. *Journal of Thermal Biology*, 25: 267 – 272.

Kettle, D.S. 1984. *Medical and veterinary entomology*. Croom Helm: London.

Klok, C.J., Chown, S.L. 1998. Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology*, 44: 615 – 628.

Klowden, M.J., Lea, A.O. 1979. Effect of defensive behavior on the blood meal size and feeding success of natural populations of mosquitoes (Diptera: Culicidae*)*. *Journal of Medical Entomology*, 15: 514 – 517.

Klowden, M.J., Blackmer, J.L., Chambers, G.M. 1988. Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. *Journal of the American Mosquito Control Association*, 4: 73 – 75.

Koehn, R.K., Bayne, B.L. 1989. Towards a physiological and genetic understanding of the energetics of stress response. *Biological Journal of the Linnean Society*, 37: 157 – 171.

Kok, D.J., Fourie, L.J., Oberem, P.T. 1994. A method for the assessment of blackfly (Diptera: Simuliidae) attraction to and engorgement on sheep. *Onderstepoort Journal of Veterinary Research*, 61: 7 –11.

Lacey, L., Escaffre, H., Philippon, B., Sèkètèli, A., Guillet, P. 1982. Large river treatment with *Bacillus thuringiensis* (H-14) for the control of *Simulium damnosum* s.l. in the Onchocerciasis Control Programme: preliminary trials with Sandoz 402 formulation. *Tropenmedizin und Parasitologie*, 33: 97 – 101.

Land Type Survey Staff. 1986. Land types of the maps SE27/20 Witdraai, 2720 Noenieput, 2722 Kuruman, 2274 Christiana, 2820 Upington, 2822 Postmasburg. *Memoirs of the Agricultural Natural Resources of South Africa* 3: 1 – 185.

Lewis, D.J., Domoney, C.R. 1966. Sugar meals in Phlebotominae and Simuliidae (Diptera). *Proceedings of the Royal Entomological Society of London* 41(10-12): 175 – 179.

Lighton, J.R.B., Quinlan, M.C., Feener Jnr, D.H. 1994. Is bigger better? Water balance in the polymorphic desert harvester and *Messor pergandei*. *Physiological Entomology*, 19: 325 – 334.

Maré, C.J. 1998. *Vesicular stomatitis: ecology of the disease in the USA. What do we know? What do we believe?* Conference Handbook Abstracts - Eighth International Conference on Equine Infectious Diseases. Dubai, United Arab Emirates, 23 – 26 March 1998.

Mason, P.G., Shemanschuk, J.A. 1990. *Black flies.* Agricultural Canada Publication 1499/E, Communications branch\Agriculture\Ottawa\K1A0C7.

Mayer, M.S., James, J.D. 1969. Attraction of *Aedes aegypti* (L): responses to human arms, carbon dioxide and air currents in a new type of olfactometer. *Bulletin of Entomological Research*, 58: 629 – 642.

McCreadie, J.W., Colbo, M.H. 1991. The influence of temperature on the survival, development, growth, and chromosome preparation quality of the EFG/C, ACD, and AA cytotypes of the *Simulium venustum-verecundum* complex (Diptera: Simuliidae). *Canadian Journal of Zoology*, 69: 1356 – 1365.

McCreadie, J.W., Colbo, H., Bennett, G.F. 1986. The influence of weather on host seeking and blood feeding of *Prosimulium mixtum and Simulium venustum/verecundum* complex (Diptera: Simuliidae). *Journal of Medical Entomology*, 23: 289 – 297.

McCreadie, J.W., Colbo, M.H., Hunter, F.M. 1994. Notes on sugar feeding and selected wild mammalian hosts of black flies (Diptera: Simuliidae). *Journal of Medical Entomology* 31(4): 566 – 570.

McIntosh, B.M., Jupp, P.G., Dos Santos, I., Barnard, B.J.H. 1980. Vector studies on Rift Valley Fever virus in South Africa. *South African Medical Journal*, 58: 127 – 132.

Merritt, R.W., Ross, D.H., Larson, G.J. 1982. Influence of stream temperature and seston on the growth and production of overwintering larval black flies (Diptera: Simuliidae). *Ecology*, 63: 1322 – 1331.

Milton, S.J. 1987. Phenology of seven *Acacia* species in South Africa. *South African Journal of Wildlife Research* 17(1): 1 – 6.

Mogi, M., Miyagi, I., Abadi, K., Syafruddin, P. 1996. Inter- and intraspecific variation in resistance to desiccation by adult *Aedes (Stegomyia)* spp. (Diptera: Culicidae) from Indonesia. *Journal of Medical Entomology*, 33: 53 – 57.

Molyneux, D.H., Davies, J.B. 1997. Onchocerciasis control: moving towards the millennium. *Parasitology Today*, 13: 418 – 425.

Myburgh, E. 1999. *Final report: Control of blackflies in South Africa.* Unpublished report to the National Department of Agriculture.

Myburgh, E, Bezuidenhout, H.B., Nevill, E.M. 2002. The role of flowering plant species in the survival of blackflies (Diptera: Simuliidae) along the lower Orange River. *Koedoe*, 44: 63 – 70.

Naksathit, A.T., Scott, T.W. 1998. Effects of female size on fecundity and survivorship of *Aedes aegypti* fed only human blood versus human blood plus sugar. *Journal of the American Mosquito Control Association*, 14: 148 – 152.

Nasci, R.S. 1991. Influence of larval and adult nutrition on biting persistence in *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 28: 522 – 526.

Nelson, G.S. 1991. Human onchocerciasis: notes on the history, the parasite and life cycle. *Annals of Tropical Medicine and Parasitology*, 85: 83 – 95.

Nevill, E.M. 1988. *The creation of permanent blackfly problems by the construction of dams. Long-term data series relating to South Africa's renewable natural resources.* South African National Scientific Programmes Report No. 157, 355pp.

Nevo, E. 1973. Adaptive variation in size of cricket frogs. *Ecology*, 54: 1271 – 1281.

Noamesi, G.K. 1966. Dry season survival and associated longevity and flight range of *Simulium damnosum* Theobald in northern Ghana. *Ghana Medical Journal*, 5: 95 – 102.

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. *Annual Review of Ecology and Systematics* 4: 25 – 51.

O'Keeffe, J.H. 1985. The blackfly problem in the Great Fish River. *The Naturalist*, 29: 3 - 8.

Palmer, R.W. 1991. *Ecological effects of impoundments in the Buffalo River, Eastern Cape, with particular reference to the distribution of blackflies (Diptera: Simuliidae).* Ph.D. thesis, Rhodes University.

Palmer, R.W. 1993. Short-term impacts of formulations of *Bacillus thuringiensis* var. *israelensis* De Barjac and the organophosphate temephos, used in blackfly (Diptera: Simuliidae) control on rheophilic benthic macroinvertebrates in the middle Orange River, South Africa. *South African Journal of Aquatic Science*, 19: 14 – 33.

Palmer, R.W. 1994. A rapid method of estimating the abundance of immature blackflies (Diptera: Simuliidae). *Onderstepoort Journal of Veterinary Research*, 61: 117 – 126.

Palmer, R.W. 1995. *Biological and chemical control of blackflies (Diptera: Simuliidae) in the Orange River.* Water Research Commission Report No. 343/1/95.

Palmer, R.W. 1997. *Principles of integrated control of blackflies (Diptera: Simuliidae) in South Africa.* WRC Report NO. 650/1/97.

Palmer, R.W. 1998. An overview of black fly (Diptera: Simuliidae) control in the Orange River, South Africa. *Israel Journal of Entomology*, 32: 99 – 110.

Palmer, R.W., De Moor, F.C. 1998. Annotated records of blackfly (Diptera: Simuliidae) distribution in southern Africa. *African Entomology*, 2: 223 – 251.

Palmer, R.W., Palmer, A.R. 1995. Impacts of repeated applications of *Bacillus thuringiensis* var. *israelensis* De Barjac and temephos used in blackfly (Diptera: Simuliidae) control, on macroinvertebrates in the middle Orange River, South Africa. *South African Journal of Aquatic Science*, 21: 35 – 55.

Palmer, R.W., Edwardes, M., Nevill, E.M. 1995a. Downstream carry of larvicides used in the control of pest black flies (Diptera: Simuliidae) in the Orange River, South Africa. *Journal of Vector Ecology*, 21: 37 – 47.

Palmer, R.W., Edwardes, M., Nevill, E.M. 1995b .Timing of larvicide treatments for the control of pest blackflies (Diptera: Simuliidae) in a semi-arid environment in South Africa. *Journal of Vector Ecology*, 21: 48 – 59.

Palmer, R.W., Edwardes, M., Nevill, E.M. 1996. Control of pest blackflies (Diptera: Simuliidae) along the Orange River, South Africa: 1990 – 1995. *Onderstepoort Journal of Veterinary Research*, 63: 289 – 304.

Parsons, D.A. 1969. A correlation between the ability to withstand high temperature and radioresistance in *Drosophila melanogaster*. *Experientia*, 25: 1000 – 1023.

Parsons, D.A. 1970. Genetic heterogeneity in natural populations *of Drosophila melanogaster* for ability to withstand desiccation. *Theoretical and Applied Genetics*, 40: 261 – 266.

Parsons, P.A. 1983. *The Evolutionary Biology of colonizing species*. Cambridge University Press: New York.

Peterson, B.V. 1959. Observations on mating, feeding and oviposition of some Utah species of black flies (Diptera: Simuliidae). *The Canadian Entomologist*, 147 – 155.

Raybould, J.N. 1967. A method of rearing *Simulium damnosum* Theobald (Diptera: Simuliidae) under artificial conditions. *Bulletin of the World Health Organization*, 37: 447 – 453.

Reeves, W.C. 1971. Mosquito vector and vertebrate host interaction. The key to maintenance of certain arboviruses. In: Fallis, A.M. ed. *The ecology and physiology of parasites.* University of Toronto Press.

Reyes-Villanueva, F., Rodrigues-Perez, M.A. 1994. Trophic status, oogenic stages, and insemination of resting populations of three *Simulium* species (Diptera: Simuliidae) in Chiapas, Mexico. *Journal of the American Mosquito Control Association* 10(3): 430 – 433.

Rodriquez-Perez, M.A., Reyes-Villanueva, F., Rodriquez, M.H. 1995. Estimating the gonotrophic cycle and survivorship of *Simulium ochraceum* (Diptera: Simuliidae) during routine vector surveillance in Southern Mexico. *Journal of the American Mosquito Control Association,* 11: 360 – 362.

Roff, D.A. 1992. *The evolution of life-histories: theories and analysis.* Chapman and Hall: New York.

Rose, M.R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster. Evolution,* 38: 1004 – 1010.

Ross, D.H., Merritt, R.W. 1978. The larval and population dynamics of five species of black flies (Diptera: Simuliidae) and their responses to selected environmental factors. *Canadian Journal of Zoology*, 56: 1633 – 1642.

Ross, D.H., Merritt, R.W. 1987. Factors affecting larval black fly distributions and population dynamics. In: *Black Flies. Ecology, Population Management and Annotated World List.* Eds. Kim, K.C., Merritt, R.W. Library of Congress Cataloging-in-Publication Data: Pennsylvania.

Samba, E.M. 1995. *Ten years of onchocerciasis control.* Report of the World Health Organization No. OCP/GVA/85.1B.

Sawabe, K., Mogi, M. 1999. Differences in energy metabolism in adult desiccation resistance among three *Aedes (Stegomyia)* species (Diptera: Culicidae) from South Sulawesi, Indonesia. *Journal of Medical Entomology*, 36: 101 – 107.

Scriber, J.M., Slansky, Jr, F. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology*, 26: 183 – 211.

Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, 24: 35 – 68.

Schmidt-Nielsen, K. 1984. *Scaling: Why is animal size so important?* Cambridge University Press: Cambridge.

Service, M. 1977. A critical review of procedures for sampling population adult mosquitoes. *Bulletin of Entomological Research* 67: 343 – 382.

Service, P.M. 1987. Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiological Zoology*, 60: 321 – 326.

Service, P.M., Rose, M.R. 1985. Genetic covariation among life-history components: the effects of novel environments. *Evolution*, 39: 943 – 945.

Service, P.M., Hutchinson, E.W., Mackinley, M.D., Rose, M.R. 1985. Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiological Zoology*, 58: 380 – 389.

Service, P.M., Hutchinson, E.W., Rose, M.R. 1988. Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. *Evolution*, 42: 708 – 716.

Shipp, J.L., Grace, B.W., Scaalje, G.B. 1987. Effects of microclimate on daily flight activity of *Simulium arcticum* Malloch (Diptera: Simuliidae). *International Journal of Biometeorology*, 31, 9 – 20.

Simmons, K.R., Edman, J.D., Bennett, S.R. 1989. Collection of blood-engorged black flies (Diptera: Simuliidae) and identification of their source of blood. *Journal of the American Mosquito Control Association*, 5: 541 – 546.

Smith, C.N., Gilbet, I.H., Gouck, H.K. 1962. Factors in the attraction of mosquitoes to hosts and their relation to protection with repellants. Proceedings of the 11th Internation Congress of Entomology Vol III: 82 – 86.

Statistica Statsoft Inc. 1991. *Statistica*, Vol I (Quick CSS). Tulsa: Okla.

Stearns, S.C. 1992. *The evolution of life-histories.* Oxford: Oxford University Press.

Steenkamp, J.A. 1972. *'n Ondersoek na die wisselwerking tussen sommige ekologiese faktore en die bevolkings van* Simulium damnosum *Theobald en* S. nigritarse *Coquillett (Simuliidae: Diptera) in die Vaalrivier by Parys.* D.Sc. thesis. Potchefstrrom University.

Stevenson, R.D. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*, 125: 102 – 117.

Struck, M. 1994. Flowering phenology in the arid winter rainfall region of southern Africa. *Bothalia* 24(1): 77 – 90.

Sutcliffe, J.F. 1986. Black fly host location: a review. *Canadian Journal of Zoology*, 64: 1041 – 1053.

Takken, W., **Klowden, M.J., Chambers, G.M. 1998**. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology*, 33: 639 – 645.

Thomas, R.H. 1993. Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. *Ecological Entomology*, 18: 84 – 90.

Thompson, B.H. 1976a. Studies on the attraction of *Simulium damnsosum* s.l. (Diptera: Simuliidae) to its hosts. I. The relative importance of sight, exhalted breath and smell. *Tropenmed. Parasit*, 27: 455 – 473.

Thompson, B.H. 1976b. Studies on the attraction of *Simulium damnsosum* s.l. (Diptera: Simuliidae) to its hosts. II The nature of substances on the human skin responsible for attractant olfactory stimuli. *Tropenmed. Parasit*, 27: 83 -90.

Toolson, E.C. Hadley, N.F. 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *Journal of Comparative Physiology*, 129: 319 – 325.

Undeen, A.H., Takaoka, H., Hansen, K. 1981. A test of *Bacillus thuringiensis* var. *israelensis* de Barjac as a larvicide for *Simulium ochraceum*, the central American vector of onchocerciasis. *Mosquito News*, 41: 37 – 40.

Usova, Z.V. 1961. The blackfly fauna of the Karelia and Murkmansk region (Diptera: Simuliidae). 286pp.

Van der Have, T.M., De Jong, G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology*, 183: 329 – 340.

Van Es, R.P., Hillerton, J.E., Gettinby, G. 1998. Lipid consumption in *Ixodes ricinus* (Acari: Ixodidae): temperature and potential longevity. *Bulletin of Entomological Research*, 88: 567 – 573.

Van Handel, E. 1985a. Rapid determination of total lipids in mosquitoes. *Journal of the American Mosquito Control Association*, 1: 302 – 304.

Van Handel, E. 1985b. Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association*, 1: 299 – 301.

Van Handel, E., Day, J.F. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in the field-*collected Aedes vexans*. *Journal of the American Mosquito Control Association*, 4: 459 – 550.

Via, S. 1993. Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? *American Naturalist*, 142: 352 – 366.

Walsh, J.F., Garms, R. 1980. The detection of plant sugars in *Simulium damnosum* s.l. by means of the cold Anthrone test. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 74: 811 – 813.

Weather Bureau. 1996. *Climate of Augrabies Falls Naional Park*. Pretoria: Weather Bureau, Department of Environmental Affairs.

Welton, J.S., Bass, J.A.B., Ladle, M., Merritt, W.J. 1987. Distribution of oviposition sites and characteristics of the egg development in the "Blandford Fly" *Simulium posticatum* (Diptera: Simuliidae). *Journal of Applied Ecology*, 24: 865 – 879.

Wenk, P. 1981. Bionomics of adult blackflies. *In*: Laird, M. (Ed.). *Blackflies. The future for biological methods in integrated control*. London: Academic Press.

Westerman, J.M., Parsons, P.A. 1973. Variation in genetic architecture at different doses of Co^{60} radiation as measured in the longevity of *Drosophila melanogaster*. *Canadian Journal of Genetics and Cytology*, 15: 289 – 298.

Williams, E.H., Williams, P.H. 1966. A note on an apparent similarity in distribution of onchocerciasis, femoral hernia and Kaposi's sarcoma in the West Nile District of Uganda. *East African Medical Journal*, 43: 208 – 209.

Winston, P.W., Bates, D.H. 1960. Saturated solutions for the control of humidity in biological research. *Ecology*, 41: 232 – 237.

Wheeler, D. 1996. The role of nourishment in oogenesis. *Annual Review of Entomology*, 41: 407 – 431.

Xue, R., Barnard, D.R. 1996. Human host avidity in *Aedes albopictus*: influence of mosquito body size, age, parity, and time of day. *Journal of the American Mosquito Control Association*, 12: 58 – 63.

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Principles of integrated control of Blackflies (Diptera: Simuliidae) in South Africa

RW Palmer

The problems caused by blackfly (*Simulium* spp.) stem from the fact that water resources are increasingly controlled as they are developed, and the natural flow variation which existed prior to control and exerted a degree of control, has been removed. The initial project investigated the use of the microbial larvicide *Bacillus thuringiensis* var. *israelensis* (B.t.i.) and an organophosphate larvicide. While these are effective, control was costly. Thus, this project set out to investigate ways of increasing the efficiency of using these methods of control by studying the relationship between population dynamics and variations in the ecosystem.

It was found that the timing of outbreaks was variable. In the lower (warmer) reaches of the river, outbreaks may occur in winter, while in the upper (cooler) reaches, outbreaks occurred in summer. A combination of high temperatures and high evaporation rates limited outbreaks during high summer. The life cycle of adults was estimated at 8 to 12 d under good conditions. One of the ways in which outbreaks could be predicted was by monitoring the population numbers. Farmers up and down the river routinely monitored adult numbers, which gave an indication of when an outbreak could be expected. At the same time, staff monitored larval numbers. Decisions to spray are based on numbers combined with other factors such as river flow and temperature. It was found that a flow at Upington of 70 cumecs was sufficient to cause problems, but the larval habitat increased significantly at flows >300 cumecs. Flow can only be used as a control between Gariep Dam and Prieska. Observation indicates that *Microcystis* blooms may be toxic to larvae, and the bivalve *Corbicula fluminalis* may limit larval numbers by competing for the same food source. While the alga *Cladophora glomerata* may play a role in natural control by providing habitat for predators, the reed *Phragmites* when trailing in the water, provides additional habitat for the larvae and hampers control.

This information was brought together in a model which assists in the decision of when to apply larvicide.

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TO ORDER: Contact **Rina** or **Judas** - Telephone No: 012 330 0340
Fax Number: 012 331 2565
E-mail: publications@wrc.org.za

Water Research Commission
Private Bag X03, Gezina, 0031, South Africa
Tel: +27 12 330 0340, Fax: +27 12 331 2565
Web: http://www.wrc.org.za

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