The influence of developmental temperature on the adult survival of

Simulium chutteri (Diptera: Simuliidae)

by

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CONTENTS

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SUMMARY

Simulium chutteri is considered a major pest in South Africa and it has been estimated that it can potentially cause stock losses amounting to more than R88 million per annum. Although a larval control programme has been launched to control the pest, major outbreaks still occur, since major fitness traits such as longevity are ignored in the planning of control actions. To improve the control programme, the aim of this study was to study the longevity of S. *chutteri* females under various conditions, and also to relate longevity to factors such as size, mass and metabolic reserves as these traits show variation that can be linked to changes in developmental temperature.

It was conclusively demonstrated in this study that the body size and mass of S. *chutteri* increases with a decrease in temperature and therefore both seasonal and geographical variations occur. A consequence of larger body size is that these individuals carry proportionally more lipid reserves than smaller ones, although these relationships were not found for glycogen.

It was shown that major seasonal variation occurred in the survival of S. *chutteri*, although these could not be attributed solely to variations in size, mass and metabolic reserves. For example, despite the large body size and mass and metabolic reserves of the winter population, it showed comparatively lower survival than all the other populations. It is argued that this is due to the interaction between fecundity and other fitness traits such as desiccation and starvation resistance. Adaptive explanations are, however, also proposed.

These results are used to explain the seasonal variation found in the annoyance levels of *S. chutteri* along the lower Orange River. Moreover, recommendations are given for the improvement of the current blackfly control programme. These include the need to control the summer population (when certain criteria are met) and showing the importance of effectively controlling the winter population.

SAMEVATTING

Simulium chutteri word beskou as 'n belangrike plaag in Suid-Afrika wat potensiële verliese in die veebedryf van meer as R88 miljoen per jaar kan veroorsaak. Alhoewel 'n beheerprogram in plek is, vind groot uitbrake steeds plaas aangesien faktore soos volwasse oorlewing ignoreer word met die beplanning van beheeraksies. Om die beheerprogram te verbeter is die oorlewing van *S. chutteri* onder verskillende toestande getoets, en is oorlewing ook bestudeer in terme van liggaamsgrootte, massa en metaboliese reserwes, aangesien hierdie veranderlikes afhanklik van ontwikkelingstemperatuur is.

In die studie is dit bewys dat *S. chutteri* se liggaamsgrootte en massa vergroot soos ontwikkelingstemperatuur verlaag. Daarom vind beide seisonale en geografiese variasie plaas. Een uitvloeisel van 'n vergroting in liggaamsgrootte is dat groter individue relatief meer lipiedes as kleiner individue dra. Hierdie verhouding is egter nie vir glikogeen gevind nie.

Dit word ook gewys dat groot seisonale verskille in die oorlewing van *S. chutteri* plaasvind, alhoewel dit nie bloot aan variasies in liggaamsgrootte, massa en metaboliese reserwes toegeskryf kan word nie. Byvoorbeeld, desondanks die relatiewe groot liggaamsgrootte, massa en reserwes van die winter populasie, oorleef hulle die kortste tydperk onder 'n reeks veranderlikes. Dit word voorgestel dat dit a.g.v. die interaksie tussen vrugbaarheid en ander faktore soos honger en uitdroging bestandheid is. Adaptiewe verduidelikings word egter ook gegee.

Die resultate van die studie word gebruik om die seisonale veranderings in die byt aktiwiteit van *S. chutteri* te verklaar. Verder word dit gebruik om aanbevelings te maak vir die verbetering van die beheerprogram, soos byvoorbeeld dat die somer populasie beheer moet word (as sekere aspekte aangespreek word) en die belangrikheid dat die winter populasie effektief beheer moet word.

1.   INTRODUCTION

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 (Fig. 1.1) 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 they 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). Furthermore, in humans, the bites of some blackfly species can cause allergic reactions known as "blackfly fever" or simuliotoxicosis (Crosskey, 1990; Palmer, 1997). This is characterized by swelling, itching, haemorrhage and oedema (De Villiers, 1987), which require 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

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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).

Fig. 1.1. Adult simuliid showing the typical humpbacked appearance of this group.

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 (Fig. 1.2) 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).

Fig. 1.2. Feeding damage caused by female blackflies to the ear of a sheep.

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 largely due to the fact that 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. 1.3).

Fig. 1.3. Map of known blackfly problem areas (in red) in South Africa

Currently 39 blackfly species are known to occur in southern Africa (Palmer, 1997; Palmer and De Moor; 1998). These include 5 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 – 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 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.

1.3) that blackfly numbers steadily started to increase along the Vaal River (De Moor, 1986) and eventually developed pest proportions (Howell and Holmes, 1969).

Since 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. After major floods in 1974, Begemann (1980) found blackflies in great numbers in the Vaal River, indicating that the ecological balance in the river had been restored, but also that the blackfly problem had not been solved.

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 spread along the lower Orange River and it soon developed pest proportions (Nevill, 1988; Jordaan and Van Ark, 1990). In 1975 the Orange Fish Tunnel, linking the Gariep Dam and Great Fish River (Fig. 1.3), 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 (Fig. 1.4) as well as forcing the larvae (Fig. 1.5) 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.
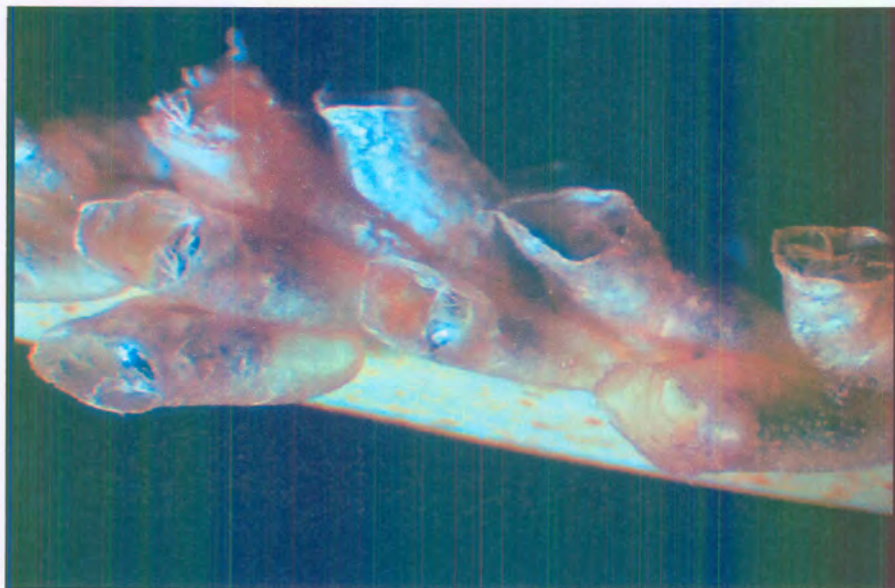
Fig. 1.4. Sessile pupal simuliids enclosed in pupal cases.
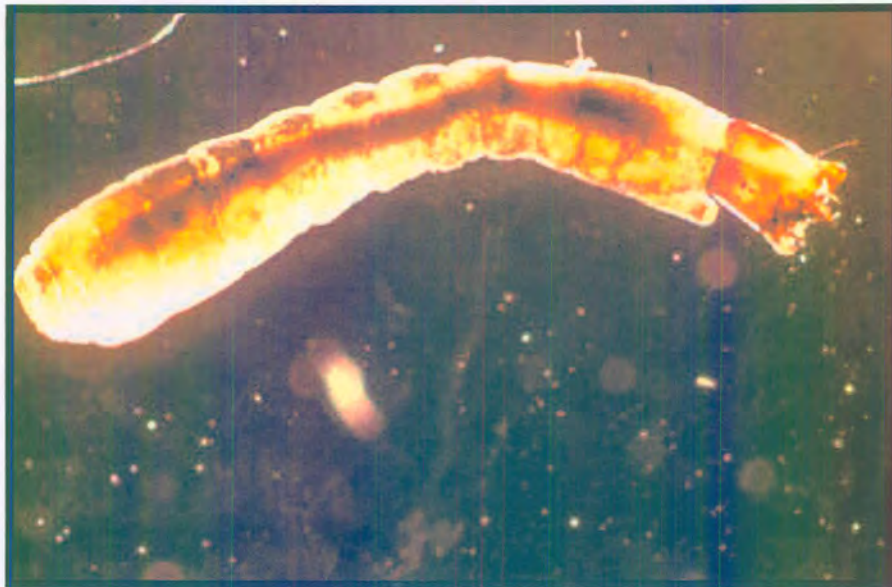
Fig. 1.5. Larval simuliid showing the strongly defined head region in comparison to the thorax and abdomen.

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 unrealistic (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 – 19 temephos and *Bti* applications are needed annually to control the pest (Palmer, 1997; Myburgh unpublished data 2000) (Fig. 1.6). For a detailed review of the ORBCP see Palmer et al (1996), Palmer (1997) and Palmer (1998).

1.4   *Purpose of the present study*

Although an effective 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.

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Fig. 1.6. Blackfly control operations along the Orange River.

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. The major goal of this study was to address these questions. 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 during seasons when conditions were less favourable.

2. BROAD DESCRIPTION OF STUDY AREA

2.1 Study sites

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.

For the purpose of this study Gifkloof (28°26'00"S; 21°23'21"E) was used as the primary study site. It is located approximately 20 km east of the Blackfly Field Station and consists of a series of small rapids (Fig. 2.2) that has been utilized as a control and research site for more than 10 years by blackfly workers. Gifkloof is excluded from all large-scale larvicide applications as it acts as a refuge for non-target organisms in the event of accidental overdosing. Prieska (29°39'38.70"S; 22°45'20.67"E) was chosen as the secondary study site. It is located approximately 200 km east of the Blackfly Field Station or 260 km upstream from Gifkloof. The Prieska site comprises a single large rapid (Fig. 2.3) that is 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 *S. chutteri* populations, from the Gariep Dam to Viooolsdrift, 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 Viooolsdrift (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). Average weekly temperatures recorded at Gifkloof from January 1991 – June 1999

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show summer highs of 26 – 28 °C and winter lows between 9 – 11 °C (Palmer, 1997; Myburgh, unpublished data, 2000) (Fig. 2.4). The Orange River has a mean discharge of 100 m³ 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 includes sheep, cattle, goats, horses, donkeys and ostriches (Department of Agriculture, Upington).

2.2   *Vegetation*

The vegetation in the study area is typical of the lower Orange River Nama Karoo Vegetation Type found within the drainage lines of the Orange River and its tributaries (Fig. 2.5). The vegetation is characterized by *Acacia mellifera*, *A. erioloba*, *Ziziphus mucronata*, *Aloe dichotoma*, *Euphorbia avasmontana*, *E. gregaria*, *Boscia albitrunca*, *B. foetida*, *Stipagrostis uniplumis* and *Tamarix usneoides* (Hoffman, 1996).
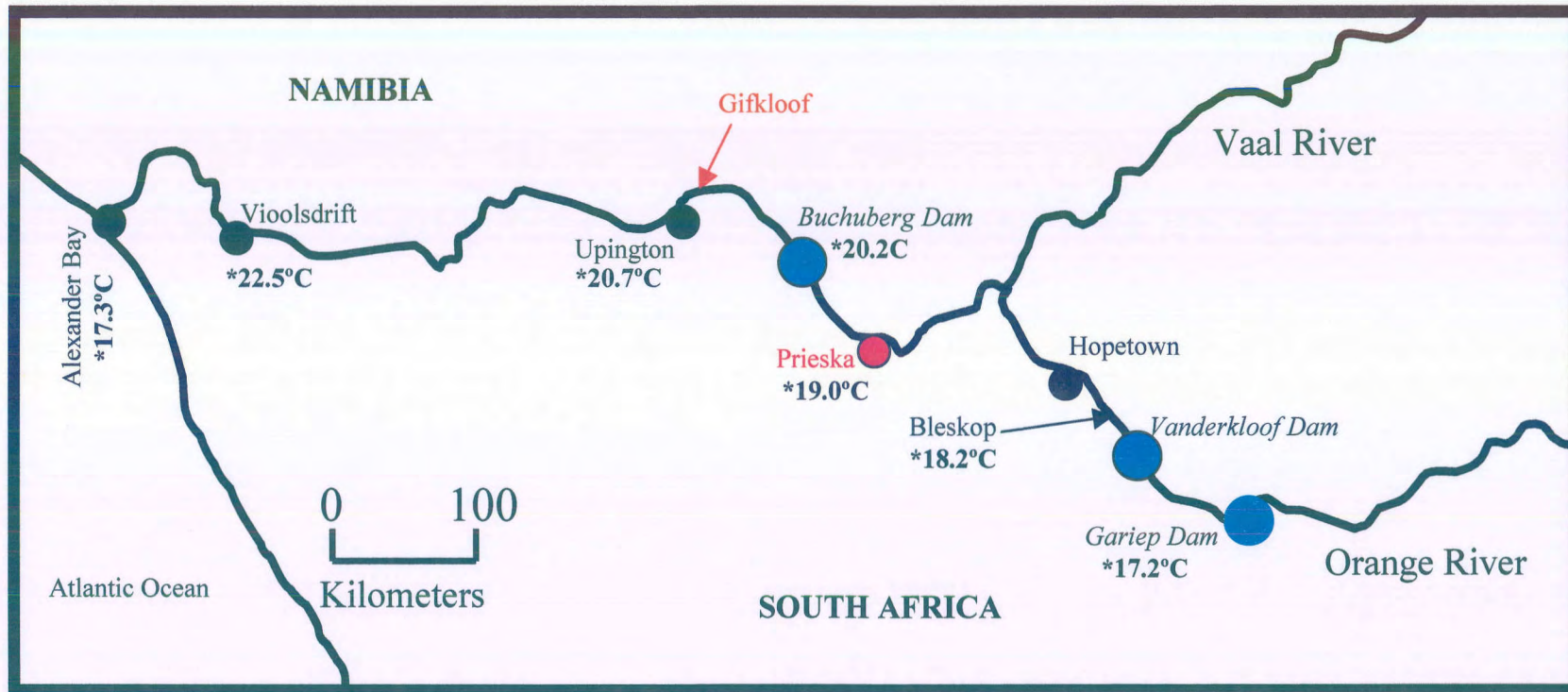
Fig. 2.1. Map of the study area showing the position of the study sites (in red), impoundments (in blue) and mean annual water temperature at various sites (Map redrawn from Palmer, 1997. Temperature data from Chutter et al, 1996; Everson, 1999).
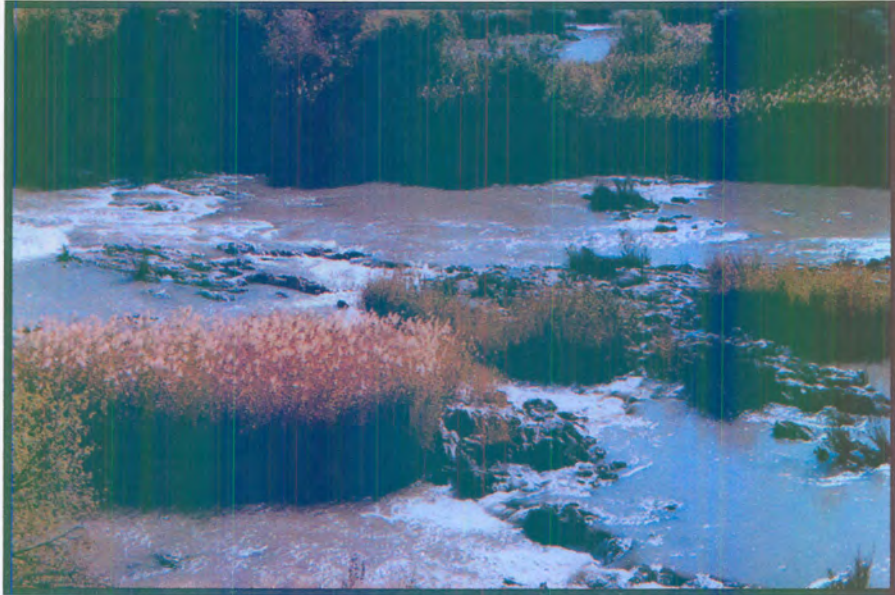
Fig. 2.2. Gifkloof - the primary study site used in this study.

Fig. 2.3. Prieska - the secondary study site used in this study.
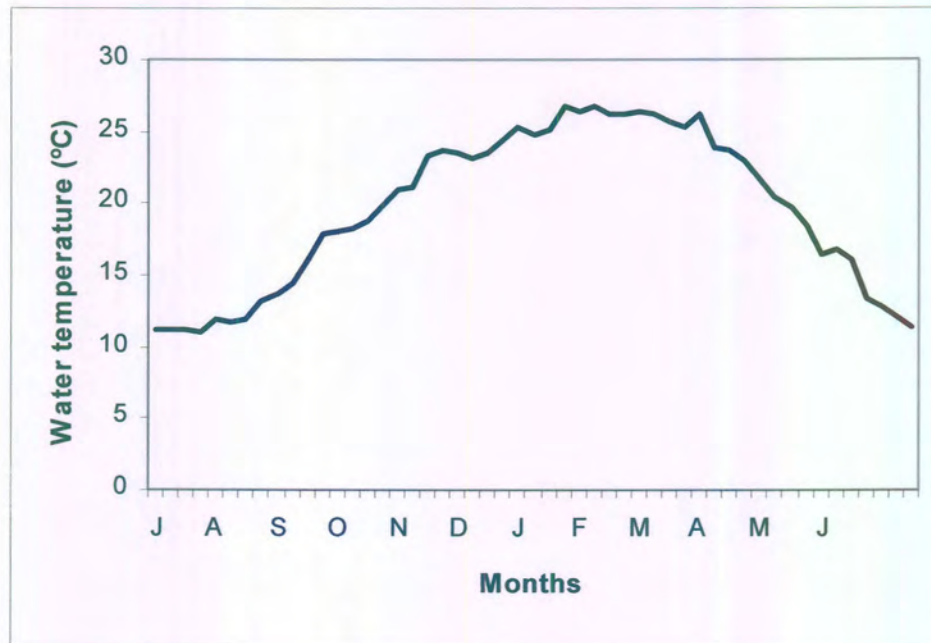
Fig. 2.4. Weekly average water temperature at Gifkloof for the period January 1991 to May 1999 (Palmer, 1997; Myburgh, unpublished data, 2000).

Fig. 2.5. Typical vegetation found along the lower Orange River.

2.3   Climate

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

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, 1997; 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. 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,

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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 (Fig. 3.1). 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

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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.
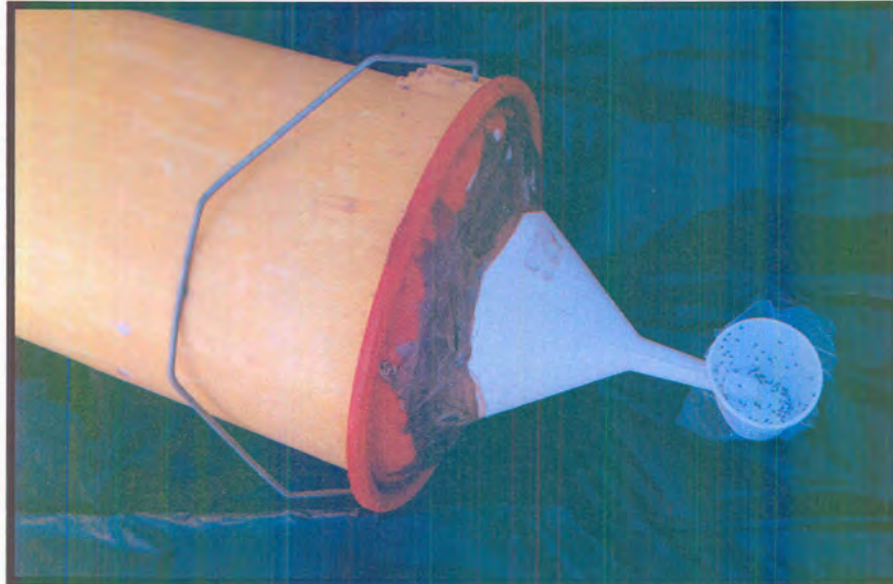
Fig. 3.1. Emergence chambers developed for the rearing of adult *S. chutteri* adults from pupae.

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. 3.2.1) 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. 3.2.2) (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).

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

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).
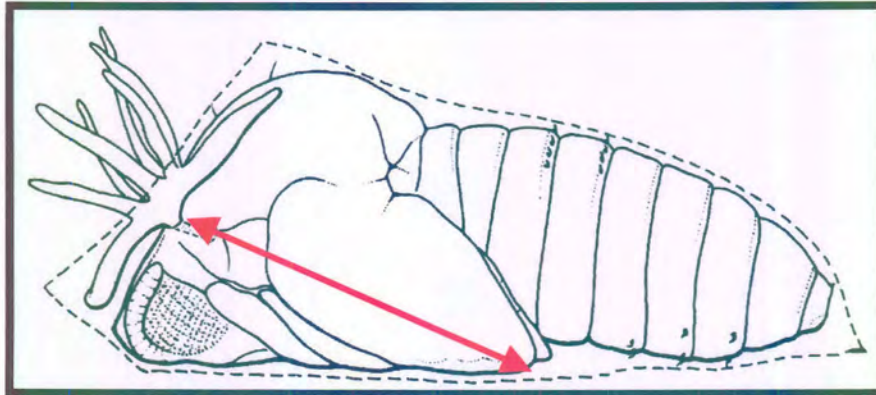
Fig. 3.2.1. Landmarks for measurement of the size of pupal *S. chutteri*.
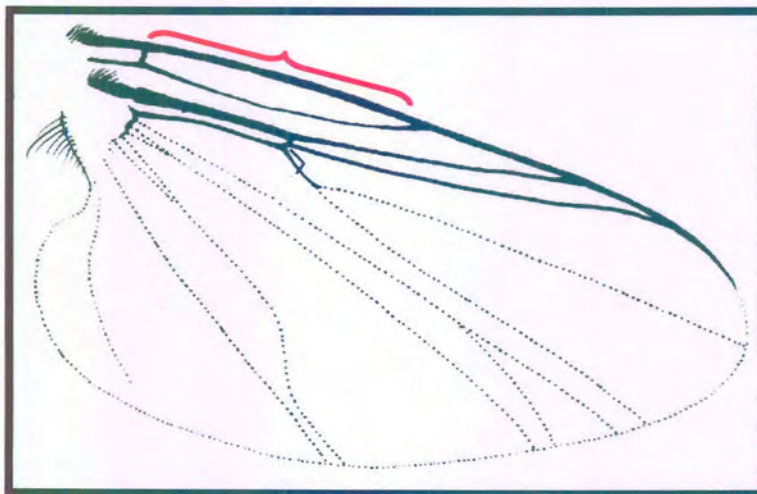
Fig. 3.2.2. Landmarks for measurement of the size of adult *S. chutteri*.

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 (1985b) 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. 3.3). 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.
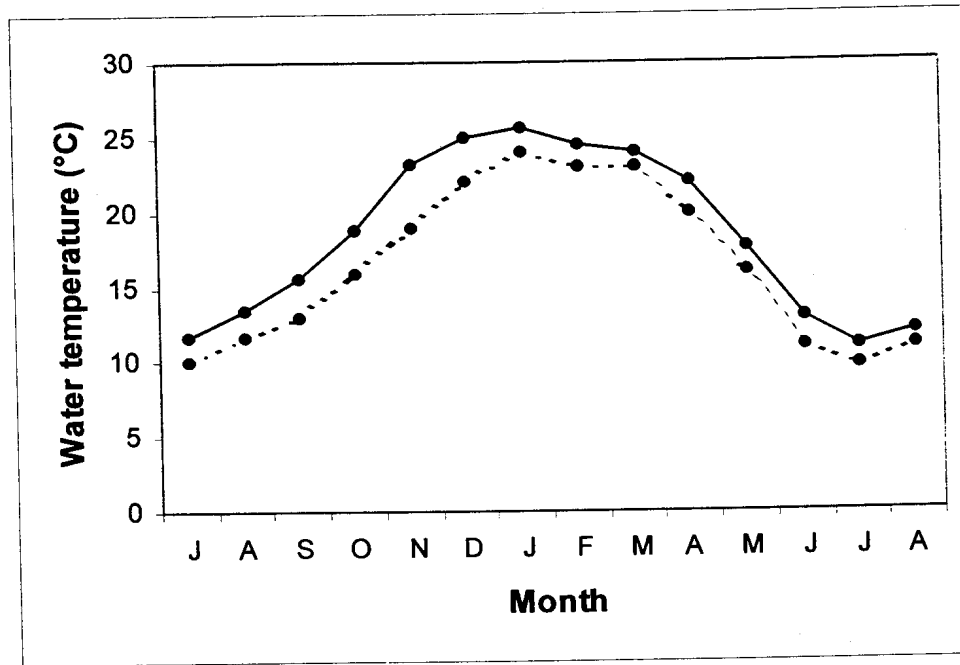
Fig. 3.3. Weekly water temperature recorded at Gifkloof (solid line) and Prieska (broken line) for the period July 1999 – August 2000.

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. 3.4.1 – 3.4.3). 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).
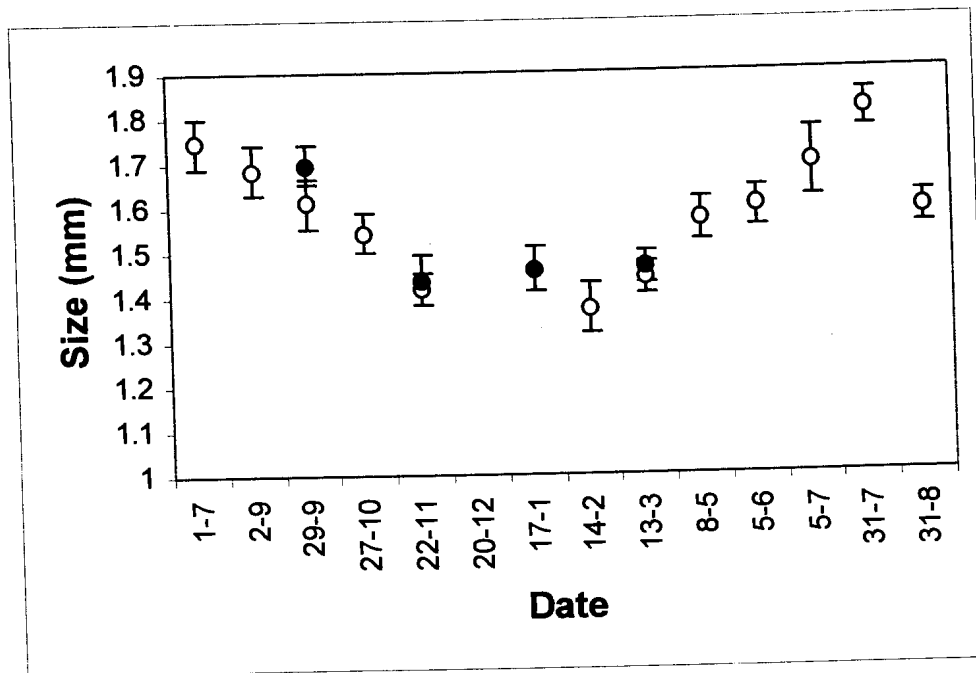
Fig. 3.4.1. Mean ± SE body size of *S. chutteri* pupae from Gifkloof and Prieska for the period July 1999 – August 2000 (Gifkloof = open symbols, Prieska = closed symbols).
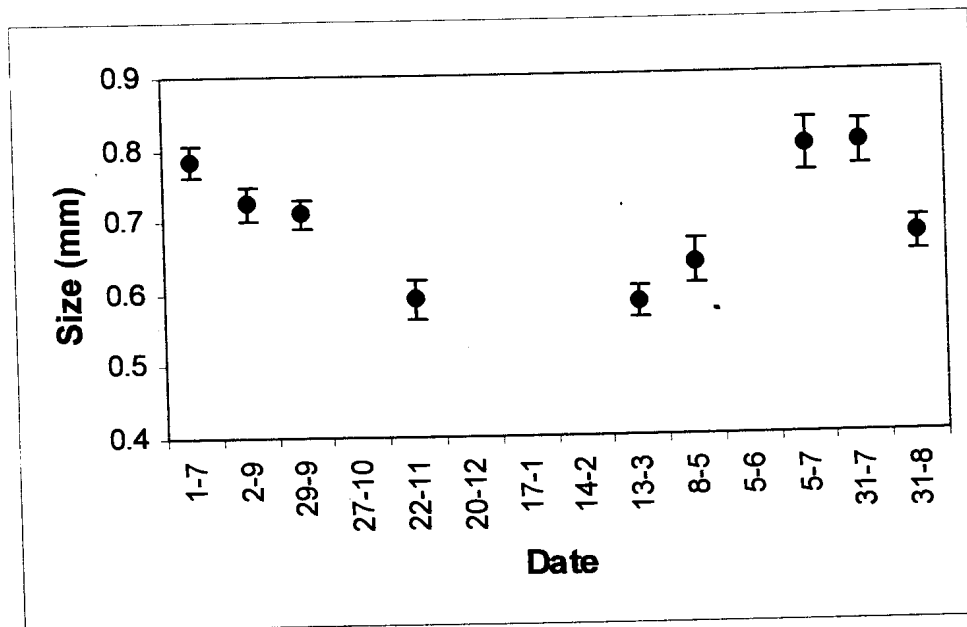
Fig. 3.4.2. Mean ± SE body size of *S. chutteri* adults from Gifkloof for the period July 1999 – August 2000.
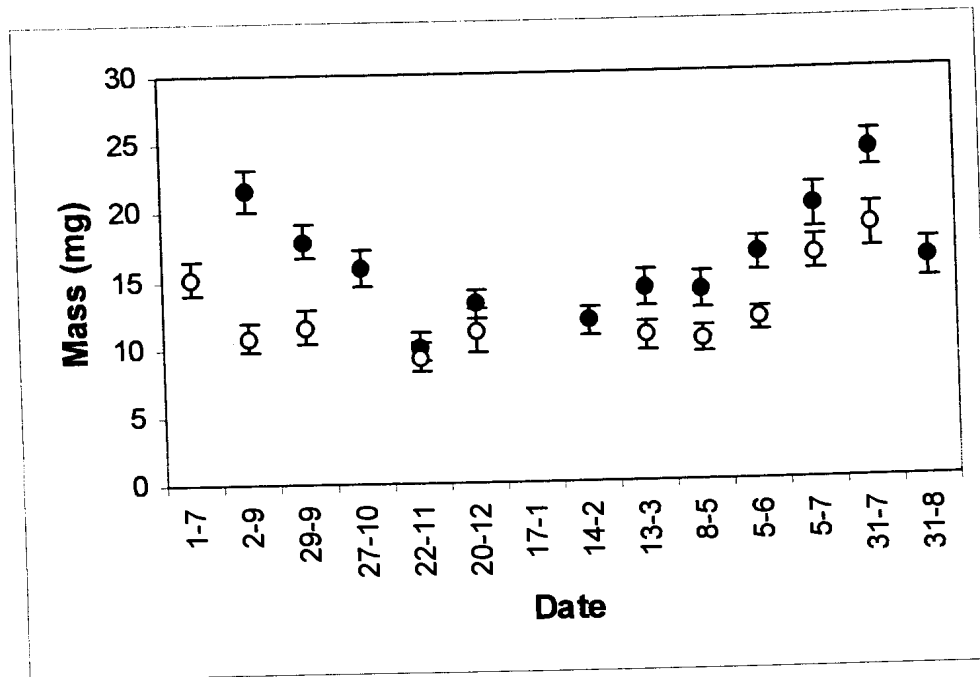
Fig. 3.4.3. Mean ± SE body mass of *S. chutteri* pupae and adults from Gifkloof for the period July 1999 – August 2000 (pupae = closed symbols, adults = open symbols).

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. 3.5), and the same was true of body mass where this was determined (Table 3.2.2, Fig. 3.5). 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).
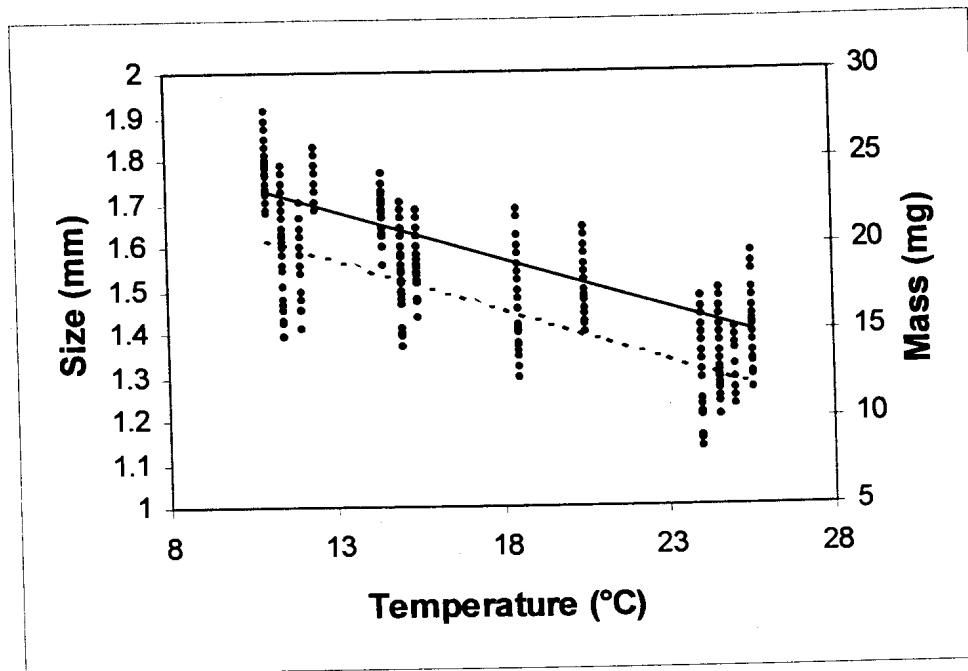
Fig.3.5. Regression plots of *S. chutteri* female pupal size (solid line) and mass (broken line) on developmental temperature.

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. 3.6.1). The percentage lipids per sample and developmental temperature were significantly, inversely related in both pupae and adults (Table 3.3.2, Fig. 3.6.2).

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. 3.6.1). In the case of percentage glycogen and developmental temperature the relationships were also not significant (Table 3.4.2, Fig. 3.6.2).

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
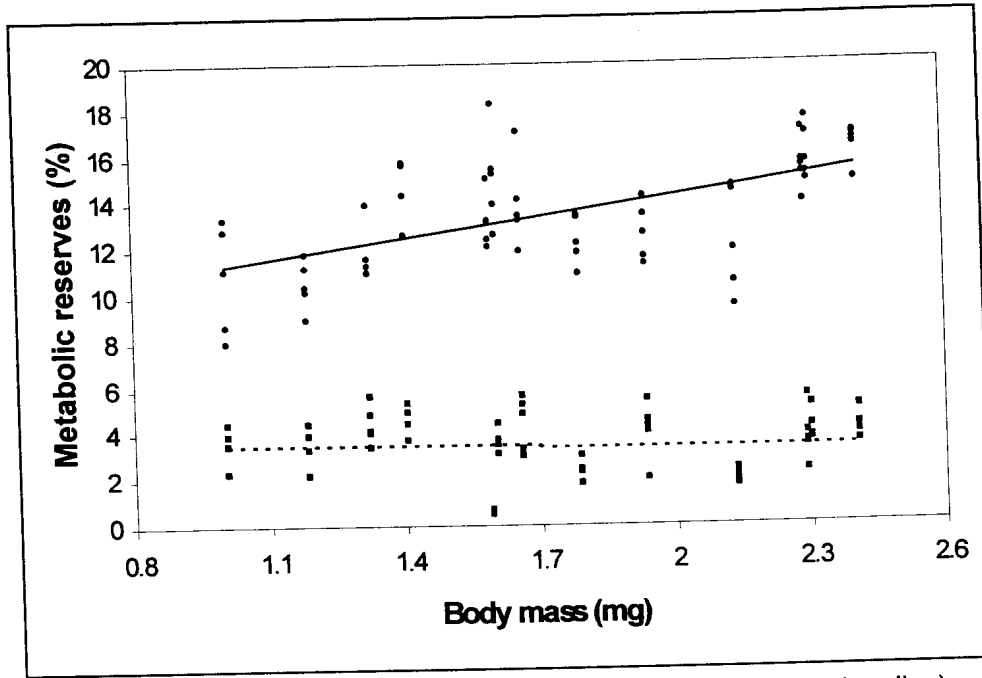
Fig. 3.6.1. Regression plots of lipid (solid line) and glycogen (broken line) content of *S. chutteri* pupae on body mass.
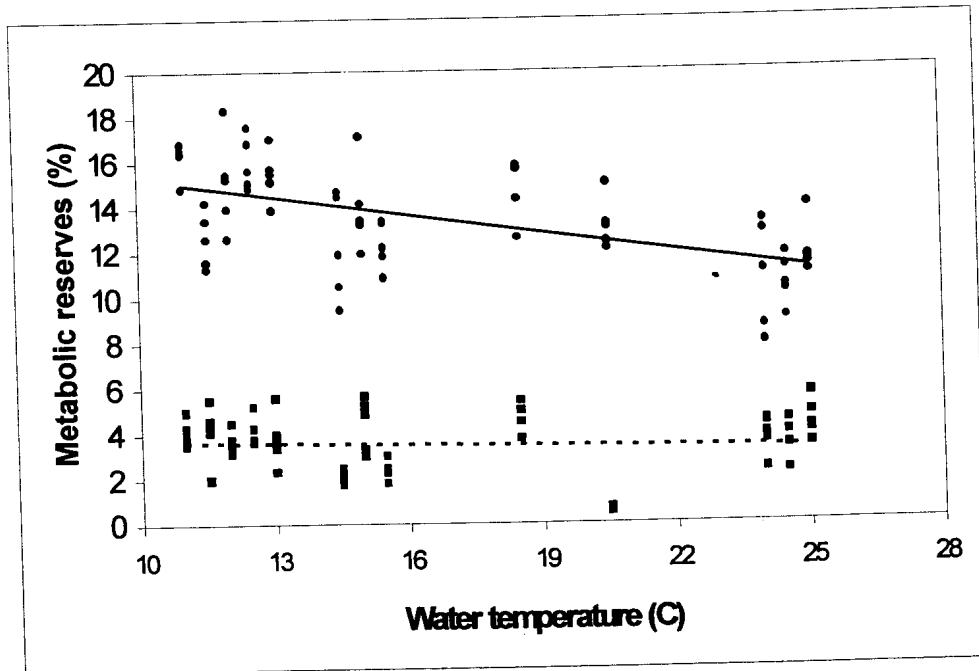
Fig. 3.6.2. Regression plots of lipid (solid line) and glycogen (broken line) content of *S. chutteri* pupae on developmental temperature.

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

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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. INFLUENCE OF TEMPERATURE, RELATIVE HUMIDITY AND STARVATION ON THE LONGEVITY OF VARIOUS *S. CHUTTERI* POPULATIONS

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). In consequence, 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 (Fig. 4.1), 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).

Fig. 4 .1. Controlled humidity chambers used in the longevity trials.

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, 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, in press). 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 (Fig. 4.1). 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	
		$LT_{50} \pm SE$ (h)	$LT_{95} \pm SE$ (h)	$LT_{50} \pm SE$ (h)	$LT_{95} \pm SE$ (h)	$LT_{50} \pm SE$ (h)	$LT_{95} \pm SE$ (h)	$LT_{50} \pm SE$ (h)	$LT_{95} \pm SE$ (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
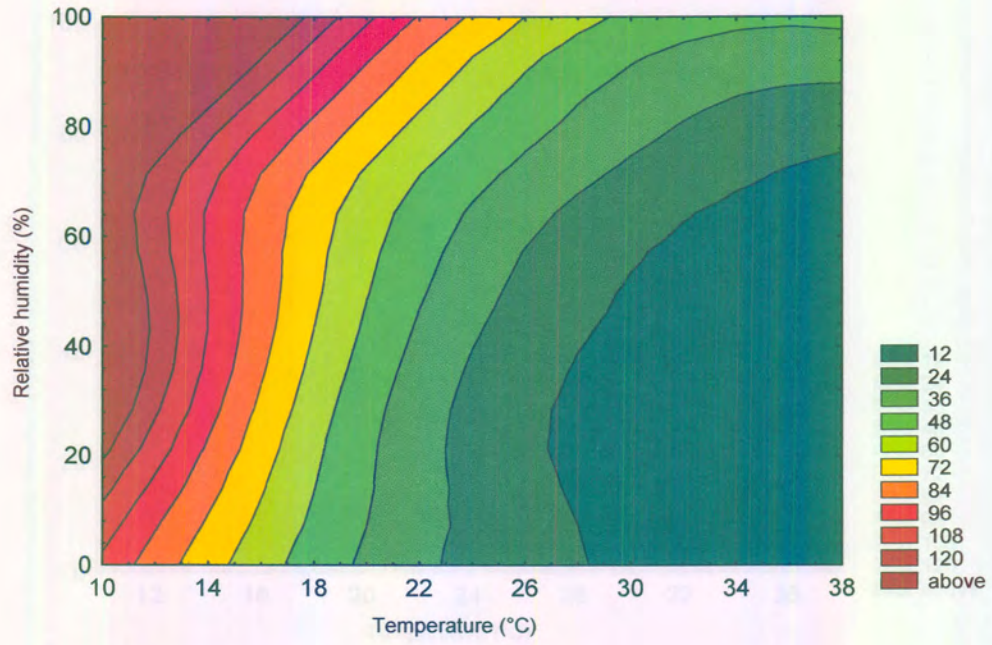
Winter population

Spring population
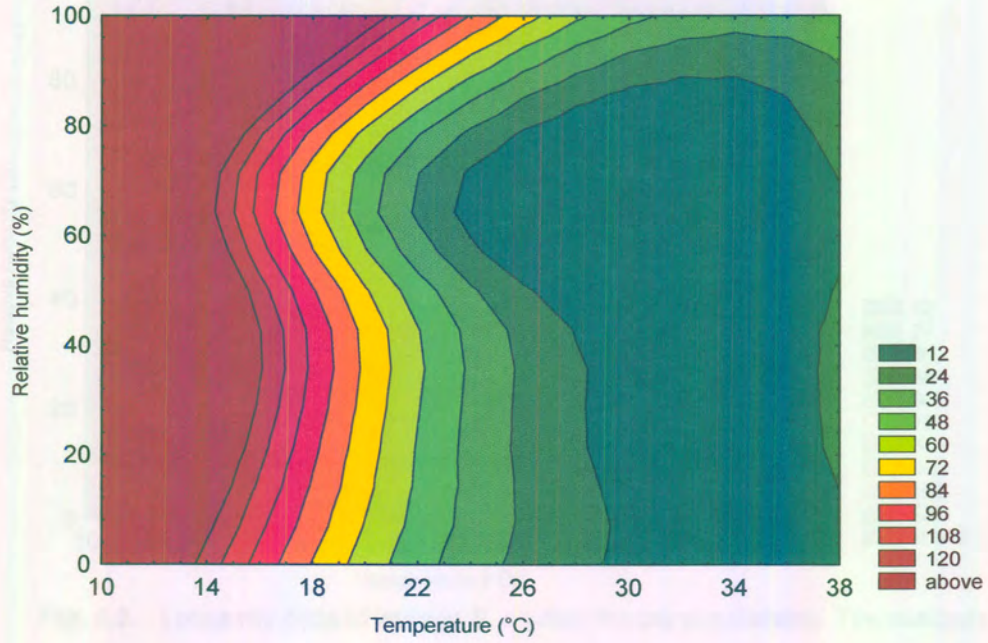
Summer population
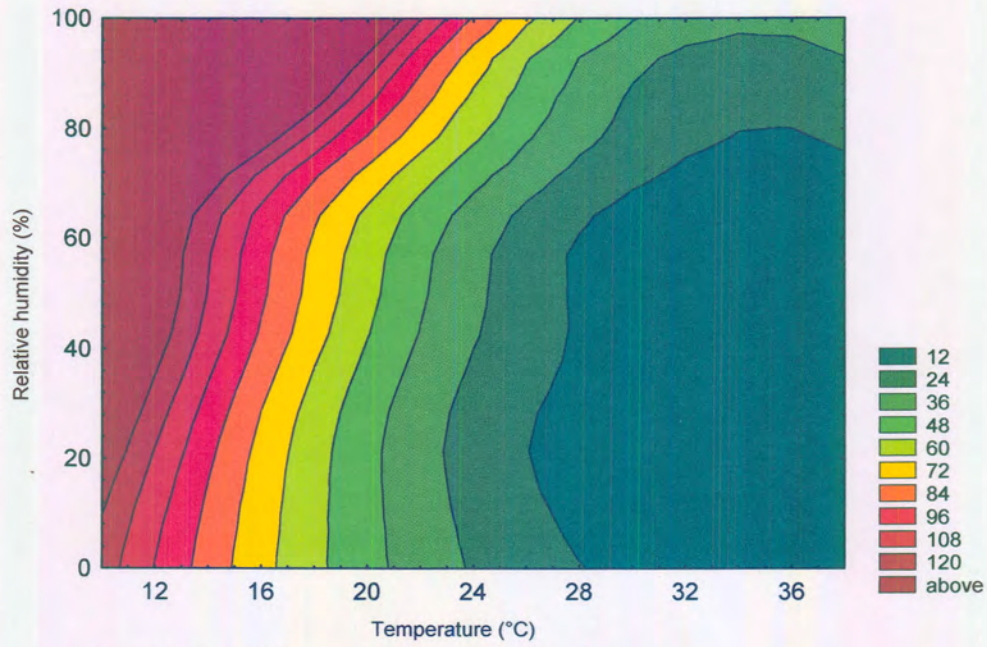
Autumn population
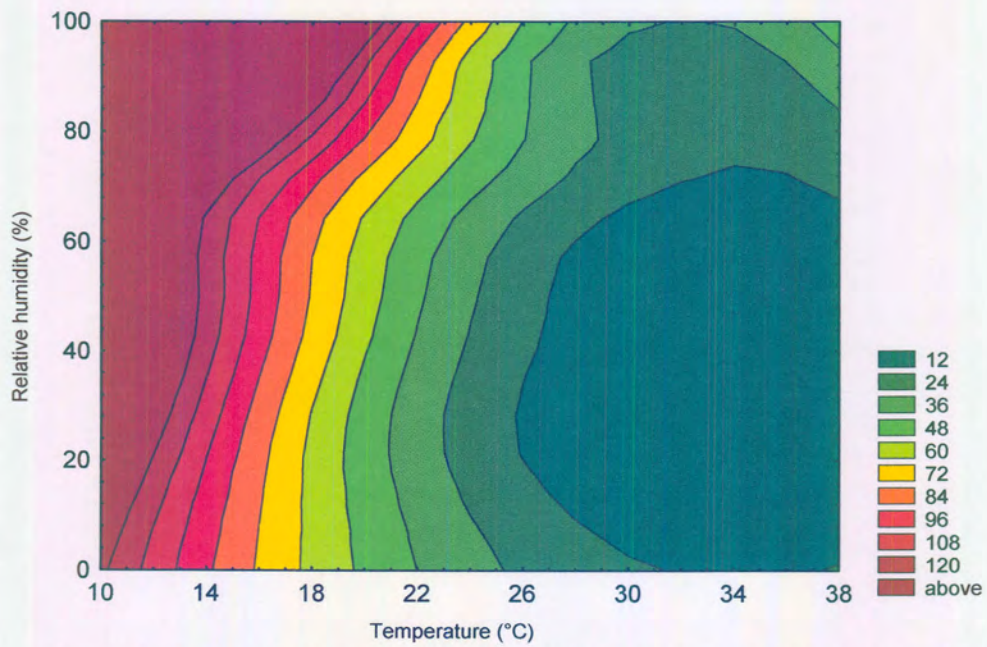
Fig. 4.2. Longevity plots of various *S. chutteri* female populations. The contours represent survival in hours

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 °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 °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°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 °C will be regarded as low, 16 – 25 °C as medium and 26 – 38 °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

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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 autogeny (i.e. high fecundity) 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.

In this chapter it was shown 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. GENERAL DISCUSSION

5.1 Introduction

Blackflies are major pests along several South African rivers and severely affect farming, tourism and recreational activities (Palmer, 1997). In South Africa *S. chutteri* is considered the most important pest blackfly species (Palmer and De Moor, 1998) and it is estimated that it can potentially cause stock losses amounting to more than R88 million per annum along the Orange River alone (Palmer, 1997). Various methods have been tested and used to control the pest, ranging from DDT applications in the 1960's (Howell et al., 1969) to water flow manipulation in the 1970's and 1980's (Howell et al., 1981, De Moor, 1982a; b) and *Bti* and temephos applications in the 1990's (Palmer, 1997).

A control programme using the latter two larvicides was launched in 1992. Although control is generally effective, periodical outbreaks still occur (Palmer, 1997). It is argued that an inability to predict the longevity of *S. chutteri* under various climatological conditions can, in part, be responsible for these outbreaks. Therefore, this present study was launched to investigate the impact that developmental temperature has on the body size, mass, lipid and glycogen content of *S. chutteri* females and how this relates to the longevity of various populations kept under different conditions.

5.2 Implications of the present study

In this study it was shown that the body size, mass and lipid content of *S. chutteri* were inversely related to 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

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summer at the highest water temperatures. Moreover, specimens developing 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, as it was shown in Chapter 3 that larger specimens will have a higher glycogen mass than smaller ones, it is assumed 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.

In this discussion it is argued that these scientifically evaluated variations in size, mass, lipid and glycogen content and longevity are important considerations in the understanding of the population dynamics of *S. chutteri*. They can also, to some extent, explain the typical seasonal variation found in the annoyance levels exhibited by adult blackflies along the lower Orange River.

5.2.1 Winter population

The winter population discussed here refers to the population hatched on 10 July 2000 that was used in the longevity trials. This population developed at 11 °C and would therefore have taken approximately 5 – 6 weeks to mature (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.).

This can be explained by the following. 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 done 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 ectotherm size/fecundity 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 increase 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 thesis, 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 then potentially lead to decreased longevity in the winter *S. chutteri* population in comparison to the others (See Chapter 4). 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 to not need to disperse away from the Orange River. In the vegetation along the Orange River temperatures are generally lower and the relative humdity higher than in the adjacent vegetation (Myburgh et al, in press). Thus, it is likely that blackflies will rather take shelter in dense 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). Thus, by not being exposed to heat, desiccation and starvation this population can potentially survive considerable periods of time in nature as predicted by the longevity plots presented in Chapter 4.

It seems that although adults are likely to be present in large numbers during winter, and have the ability to live 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.

5.2.2. Spring population

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

Along the Orange River the largest outbreaks usually occur during spring each year (Jordaan and Van Ark, 1990; Palmer, 1997). This present study showed that many factors can potentially be 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).

In addition to the above-mentioned 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). This, together with the fact that one of their favourite, abundant and widespread nectar sources, *Acacia mellifera* (Myburgh, et al, in press) flowers at this time of the year, will furthermore 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.

5.2.3. 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 developmental period (7 – 10 days) (Palmer, 1997) associated with the high developmental temperature (26 °C). In this discussion the summer population refers to adults hatched on 10 January 2001.

It is hypothesized, although not yet satisfactorily proven, that blackfly numbers are generally low during the summer months because the climatological conditions experienced at that time of the year severely limit adult survival (Palmer, 1997). Despite this, major outbreaks do occur periodically during the summer months, which suggests that *S. chutteri* do indeed posses the ability to be a nuisance at that time.

In the present study, it was shown 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 was less than 24 h at temperatures exceeding 27 ^{0}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, in fact, be severely limited at that time. As this population also emerged with low metabolic reserves they would need to take a sugar meal as soon as possible 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 populations are capable of utilizing other mechanisms than those studied here to overcome the harsh conditions or are favoured by environmental conditions during certain years such as high flow conditions or low ambient temperature.

5.2.4. 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 developmental period of 2 – 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 months. This can in part be attributed to the fact that the individuals that developed during autumn is 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.

5.3 *Implications for the control programme*

5.3.1 Present control actions

It is currently recommended that blackfly control operations along the lower Orange River are only necessary from July – October and from March – April (Palmer, 1997), owing to the increase in adult annoyance during these periods. However, this recommendation has been questioned in recent years because periodic outbreaks have occurred during mid-summer.

5.3.2. The need for summer larvicide applications

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 as this time. However, as outbreaks do occur over the summer months, either *S. chutteri* are capable of using other mechanisms to overcome these harsh conditions, or outbreaks are restricted to cooler years of higher rainfall. Thus, it is recommended that larvicide applications can be considered during the summer months, but only after and when:

1. 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;

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.

5.3.3. Importance of the winter population

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 this population before it emerges and lays eggs, especially as it may be autogenous.

5.3.4. Application of longevity plots

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.

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