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Assessment of blackfly (Diptera: Simuliidae) problem status and potential biological control agents along the Vaal and Orange Rivers in South Africa.

by

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SUMMARY

Blackflies (Diptera: Simuliidae) are major pests in the livestock and labour-intensive farming systems along the major rivers in South Africa. At present, blackflies are controlled with the larvicide *Bacillus thuringienses* var. *israelensis* (*B.t.i.*). As part of establishing an environmentally friendly and cost-effective Integrated Pest Management (IPM) program against blackflies, investigations were initiated to support the present blackfly-control strategy in South Africa. Emphasis was placed on potential predators and parasites of the blackflies' aquatic stages.

Questionnaires were presented to livestock farmers along the Vaal and Orange Rivers to determine public views concerning blackfly annoyance. Furthermore, blackfly populations at thirteen sites along the Orange River, twelve along the Vaal River and one site along each of two tributaries to the Vaal River, namely the Riet and Harts Rivers, were monitored seasonally for one year. The abundance of the aquatic stages of blackflies and potential predators on stones and vegetation in the river was determined using the 10-point visual ranking system of Palmer (1994) and the South African Scoring system (SASS 5), respectively. The abundance of algae as well as other environmental factors, namely water flow, water temperature and turbidity, were also monitored.

Farmers who were contacted along both the Vaal and Orange rivers indicated that they experience severe blackfly problems during the summer months and that the majority of farmers were not aware of any products available that could protect their animals against blackfly attacks. The farmers also indicated that they were willing to be involved in blackfly research to improve this situation.

Blackfly larvae and pupae were found in high abundance in both the Vaal and Orange Rivers.
Simulium chutteri was the most abundant species in the Orange River and *S. adersi* in the Vaal River.

In both the Vaal and Orange Rivers there was no significant correlation between immature blackfly abundance and water flow and turbidity. Water temperature also played a role in the seasonal build-up of blackflies in the winter months.

The three most abundant algae classes were Bacillariophyceae, Chlorophyceae and Cyanophyceae. Cyanophyceae was the only algae group that had a negative correlation with blackfly immature abundance in the Orange River; this was not statistically significant. There were no negative correlations in the Vaal River.

In both the Vaal and Orange rivers, blackflies were infected with Mermithidae nematodes and Microspora protozoans. In the Vaal River, the infection prevalence in natural conditions was the highest for Microspora and in the Orange River the highest for Mermithidae.

The most important families of blackfly predators identified were Hydropsychidae and Gyrinidae. Hydropsychidae was the only family recorded in high abundance but this predator had no effect on abundance of immature blackfly. Gyrinidae gave a negative correlation with immature blackfly abundance; however, this was not significant.

The biological control agents identified in this research need to be evaluated further for use in an IPM approach with the current control system, *B.t.i.*

1. INTRODUCTION

1.2. Background

Blackflies are in the Order Diptera and Suborder Nematocera (Crosskey, 1986; Crosskey, 1990). The largest and most important family is the Simuliidae. According to Kettle (1992) the most important genus is *Simulium* with 1000 species arranged in 38 subgenera. The females of some species are haematophagous and it is this feeding activity of the female that makes blackfly species vectors of disease and pests. Although blackflies are not known to transmit any diseases in South Africa, they are regarded as a pest due to their “nuisance” and biting attacks.

The interaction between blackflies and man can be dated back to 1604, when an explorer by the name of Samual de Champlain encountered them while he and his party were building a defensive barrier on Saint Croix island (Crosskey, 1990). The blackfly adults were described first and thereafter the pupa in 1784 (Crosskey, 1990). The larva had been identified much earlier but the connection between the larva and the life cycle was only made in 1822 (Crosskey, 1990). The egg was probably first discovered in 1843 (Crosskey, 1990). Through history it was evident that the nuisance caused by blackflies was always problematic. Some relief could be found by lighting smoke-fires, known as smudges, in eastern Canada and the northern United States. The Second World War, however, was a turning point in the necessity to control arthropods of medical end veterinary importance. This gave way to research into insecticidal control. Field trials with DDT started in 1944 and it became evident that this insecticide could also be used to control blackflies (Crosskey, 1990).

1.2. Morphology

The adult blackfly is small, with a body size of about a match head (Fig. 1.1a, b). It has a sturdy body, however, with the head attached low on a typically humped thorax (de Moor, 2003). This characteristic led to the name Buffalo-gnat (Crosskey, 1990). It has a pair of eleven-segmented, cigar-shaped antennae and toothed mouthparts (de Moor, 2003). The proboscis is shorter than the height of the head and directed downwards (hypognathous) (Crosskey, 1993)

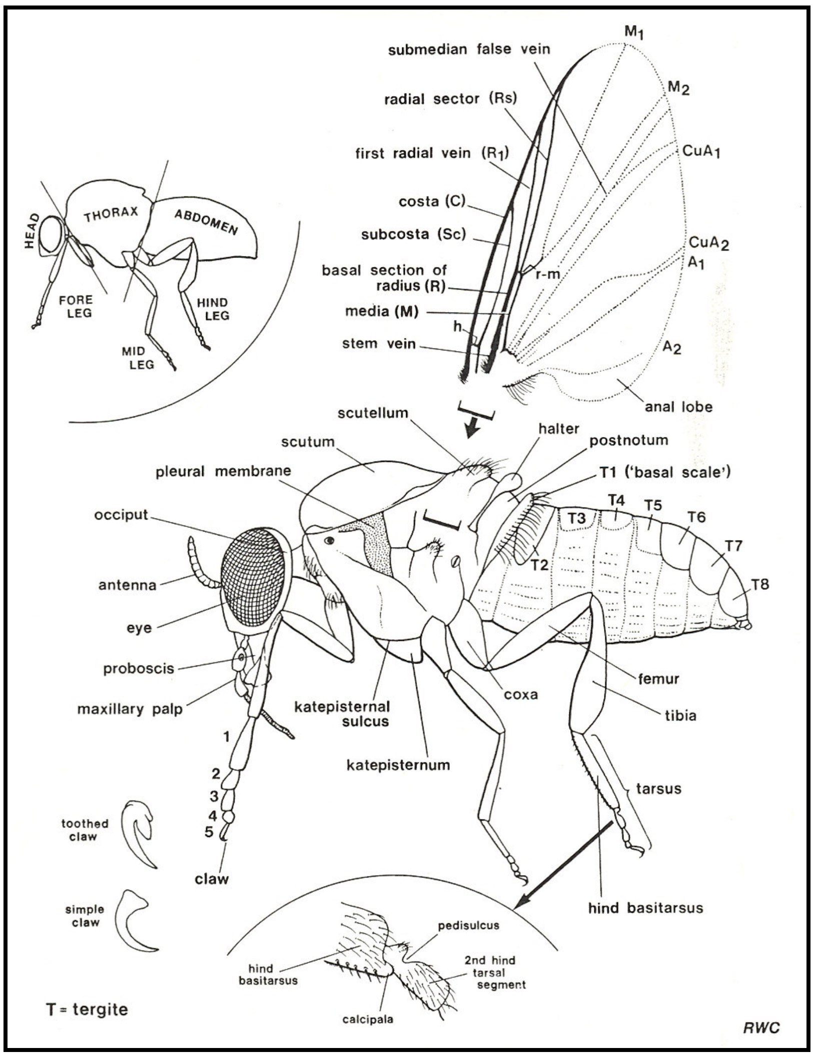


Fig. 1.1A Basic morphology of the adult female *Simulium* (Crosskey, 1993).

Fig. 1.1B Blackfly Adult (Photograph by John Putterill, Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI)).

The adult also has two large, rounded wings that fully overlap the abdomen when at rest (de Moor, 2003). The legs are often all black but they can be yellow and black in some species; also the legs have five tarsal segments and paired tarsal claws (Crosskey, 1993). The females' abdomens are mostly membraneous as they need to compensate for large blood meals (Crosskey, 1993). The males and females can be distinguished by their eyes. The males (Fig. 1.2A) have eyes that meet in the centre and have large facets on the top half and smaller on the bottom. The females (Fig. 1.2B) have well separated eyes with uniformly sized facets (de Moor, 2003). These adaptations of the males' eyes are probably linked to their mating activity (de Moor, 2003).

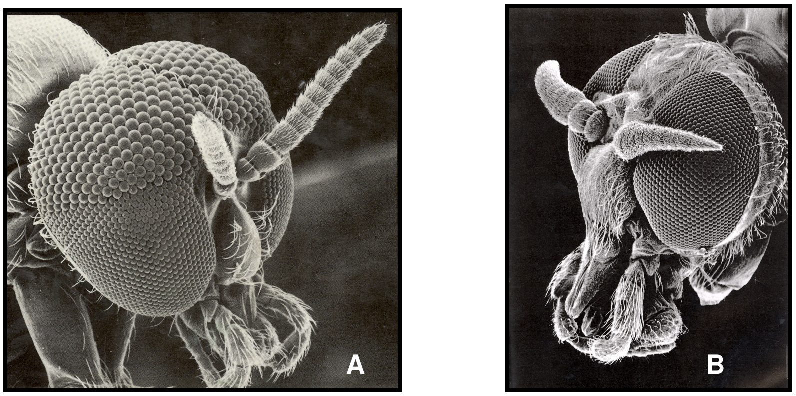


Fig. 1.2 Head of the male (A) (Crosskey 1990) and Female (B) blackfly (Photograph by Doug Craig, University of Alberta, Canada).

The larva is wormlike; the segmentation between the thorax and abdomen is not clearly visible (Fig. 1.3) (Crosskey, 1993). It also has a posterior swollen abdomen and has no legs (Crosskey, 1990; Howell and Holmes, 1969). The larva has a pair of cephalic fans used in feeding. Apart from this, it also has a labrum overhanging the mouth entrance, paired mandibles and maxillae laterally (Crosskey, 1993; de Moor, 2003). The mandibles have teeth and brushes (Crosskey, 1993). The larva also has two pseudopods known as prolegs (Crosskey, 1990). One is positioned immediately behind the head, while the other forms the posterior end of the body (Crosskey, 1990). The posterior proleg has hooks arranged in a circular format, which is used for attachment (de Moor, 2003). The older larva has buds of the adult wings and legs and the pupal gill (Crosskey, 1993).

Fig. 1.3 Blackfly larva (Photograph by John Putterill, ARC-OVI)

The pupa (Fig. 1.4) is of the obtect type. It is embedded in shoe- or slipper-shaped silken cocoons (Fig. 1.5A, B). The shape is associated with the location of the pupa in either fast or slower flowing water (Crosskey, 1993; de Moor, 2003). They also have thoracic respiratory organs (spiracular pupal gills) that are often branched and enable them to breathe under water (de Moor, 2003). The diversity in the form of the respiratory organs makes Simuliidae unique among insects (Crosskey, 1993).

Fig. 1.4 Blackfly pupae on vegetation (Photograph by John Putterill, ARC-OVI).

A B

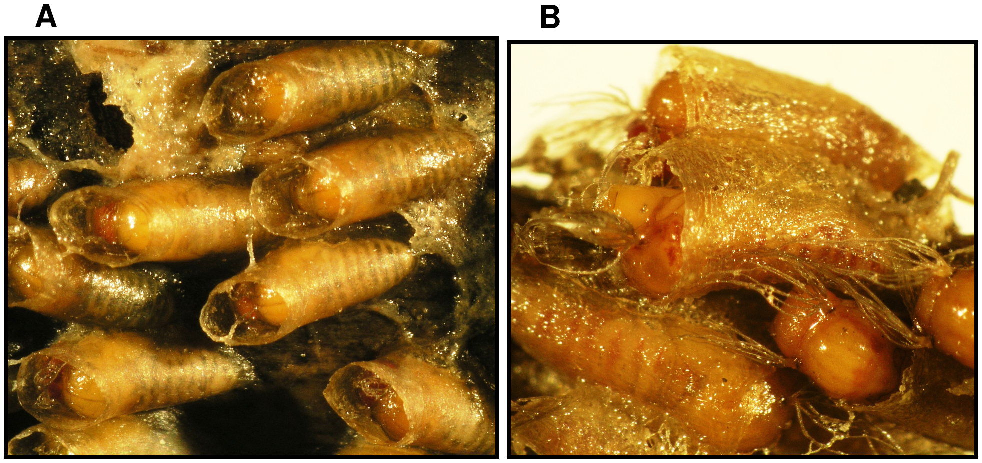


Fig. 1.5 Pupae embedded in silken shoe-shaped (A) or slipper-shaped (B) cocoons (Photograph by John Putterill, ARC-OVI).

1.2. Biology

Figure 1.6 shows that the life cycle of blackflies passes through four developmental stages: egg, larva, pupa and adult.

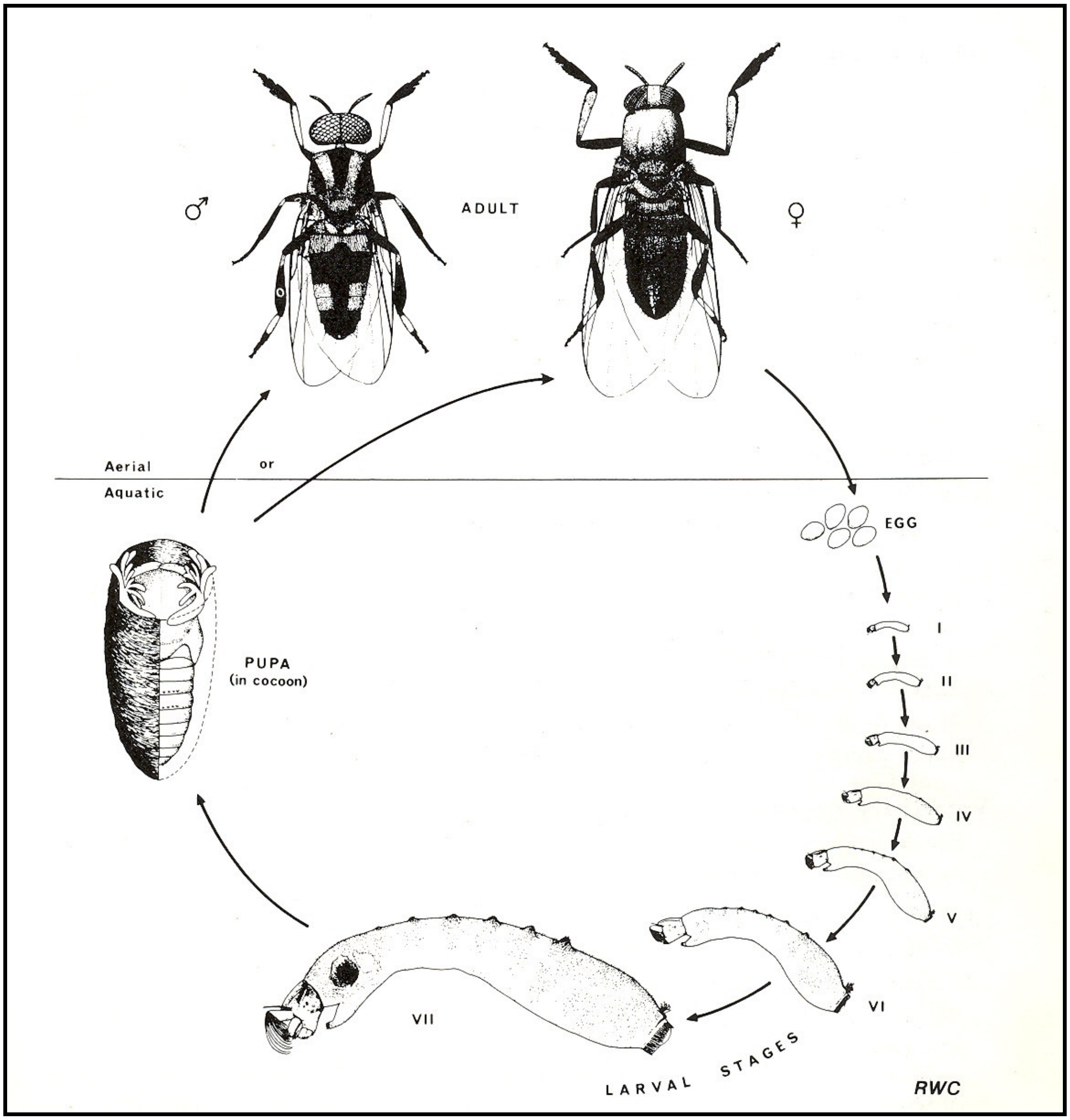


Fig 1.6 Illustration of the blackfly life cycle (Crosskey, 1990).

1.2.1. Adults and eggs

On a calm and windless day, males of the same species will form mating swarms. These swarms can be found directly above the water or in the vicinity of the host animal (de Moor, 2003; Howell and Holmes, 1969; Palmer, 1997). Mating takes place when the females become visible to the males in the swarm, usually as the females emerge from the water (de Moor, 2003; Palmer, 1997). Copulation starts in flight where after the adults fall to the ground and copulation continues on solid surfaces (Crosskey, 1990; Palmer, 1997).

Adult blackflies, both male and female, feed on sugars that provide them with carbohydrates for longevity and energy to fly (Davies *et al.*, 1962; Burgin and Hunter, 1997; Myburgh *et al.*, 2001). Female blackflies of some species are also blood feeders. These blood meals provide the female with proteins that certain species of blackfly require in the development of eggs (Braverman, 1994; Howell and Holmes, 1969; Palmer, 1997). After feeding, the female usually rests and then returns to the river to lay her eggs (Palmer, 1997).

The eggs can be found in clusters on rocks, in stream vegetation or other objects below the water surface, usually in masses of 100 to 500 (de Moor, 2003; Freeman and de Meillon, 1953; Palmer, 1997). Some species lay the eggs on the water surface in flight. These eggs then settle in the sediment (Palmer, 1997). The development rate of blackfly eggs is highly variable and can be categorised into five stages, as described in Palmer (1997). According to Crosskey (1990), the minimum development time of blackfly eggs is two days. This development time is dependent on water temperature (Palmer, 1997).

2.2.1. Larvae and pupae

According to de Moor (2003), the blackflies' best adaptation is that the larval and pupal stages inhabit flowing water. This adaptation limits the distribution of blackflies. One of the larval adaptations is that it can change its attachment site, to a more suitable site in faster current velocity (de Moor, 2003; Hansen *et al.*, 1990; Palmer, 1997). According to Hansen *et al.* (1990), microhabitats with high velocities (water flow rates) can provide larvae with a refuge to protect them from predators. Because of this advantage, larvae prefer faster flowing water. Larvae have a further defensive reaction: when in danger they can detach from their attachment site and hang on a silk thread; when the danger has passed they can re-attach to the original site (de Moor, 2003; Palmer, 1997).

The larvae of most blackfly species are filter feeders (Braverman, 1994). Thus, blackflies are seen as biological filters and can feed on diatoms, algae, microscopic invertebrates and bacteria (de Moor, 2003). Most blackfly larvae develop through seven instars and the final instar is known as a pharate pupa (Palmer, 1997). Before the pharate pupa can complete pupation it spins a silken cocoon. As seen above (Fig 6), this cocoon can be either slipper- or shoe-shaped and this characteristic is species specific (de Moor, 2003). A set of hooks on the abdomen of the pupa keeps it firmly inside the cocoon where it is immobile (De Moor, 2003). The pupa also has outward extending gills that function as respiratory organs (Braverman, 1994; Howell and Holmes, 1969).

The developmental rate of pupae is rapid, because at this stage the insect is vulnerable to predation and changes in water level. After pupation, the adult fly emerges from the cocoon and floats to the water surface in an air bubble (Palmer, 1997). It then rests on nearby vegetation to allow its wings to harden (Palmer, 1997).

According to Palmer (1997), consistent seasonal trends can be seen in larval abundance in the Orange River. Larval abundance is lowest in March and April (autumn) and highest in August (late winter) and September (spring). The consistent build-up of larval numbers through winter suggests that larval development at this time of the year is slower than the hatching of eggs

(Palmer, 1997). Blackflies can also undergo diapauses in the egg, larval, pupal and adult stages (Palmer, 1997) and this can influence any control strategy.

1.2. Blackfly importance in South Africa

Blackflies are at present seen as major pests in the livestock and labour-intensive farming systems in South Africa (Palmer *et al*., 1996; Palmer, 1997). In 1962, when construction of several dams in the Orange River were announced as part of the Orange River Development Project , there was concern about what impact this may have on the downstream reaches of the river (Van Vuuren, 1992). Nevill (1988) stated that blackflies had not been seen as pests in South Africa, but reached pest status after the construction of dams, canals, irrigation schemes or hydro-electrical plants along rivers. The rivers in South Africa that are known to have blackflies as pests are the Orange, Vaal, Great Fish, Sundays, Olifants, Berg, Eerste and Gamtoos Rivers (Fig. 1.7) (Palmer, 1997; Myburgh and Nevill, 2003).

Along the region of the Orange River, the blackfly, *Simulium chutteri* Lewis, is one of the most important pest species. *Simulium chutteri* has been recorded to feed on the blood of sheep, horses and ostriches (Howell *et al*., 1981; Palmer, 1997). Blackfly bites are painful. The bites also irritate the skin and this can result in localised bacterial infection (Fig. 1.8) (Molloy, 1990). Allergic reactions to blackfly bites have also been found to lead to the death of cattle (Crosskey, 1990).

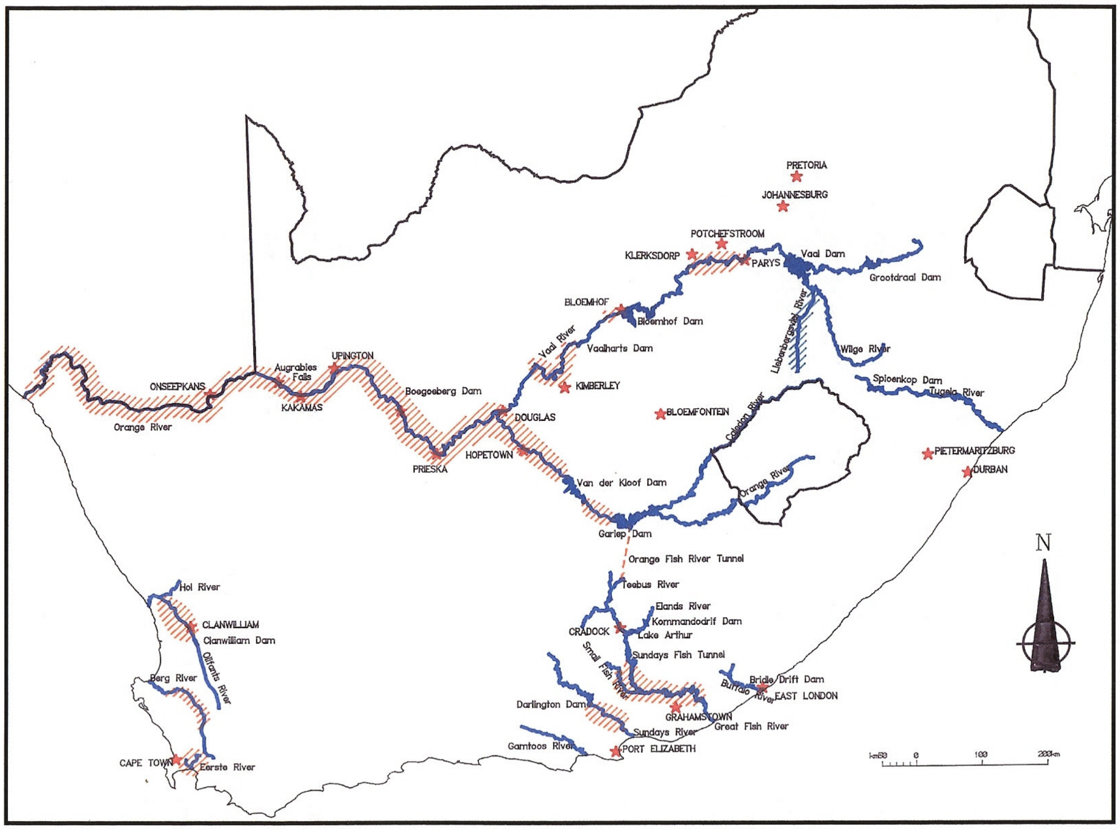


Fig. 1.7 The extent of the blackfly problem in South Africa (Palmer, 1997).

Fig. 1.8 Skin irritation and damage on the ear of a sheep caused by the biting of blackflies (ARC-OVI Archives).

The main effect of blackfly attack is irritation, which can affect the grazing and mating of sheep. As soon as they detect blackflies, sheep run and huddle together and do not feed. It is this secondary effect and its consequence which causes the main economic losses to stock farmers. According to Palmer *et al.* (1996), annual losses in sheep production along the Orange River was estimated by the Northern Cape Agricultural Union to be R88 million. In 2005, Conningarth Economists did an economic survey along the Orange River below Hopetown and determined annual sheep production losses of nearly R30 million (Bath, 2006 unpublished report, University of Pretoria). Other livestock and industries are also affected, however. The main industries and organisations affected by blackflies in South Africa are: stock farming, dairy farming, poultry farming, ostrich farming, labour-intensive farming (e.g. orchards and vineyards), irrigation farming, Department of Water Affairs and Forestry, wildlife, tourism and recreation as well as diamond mining (Palmer, 1997).

Blackflies do not act as vectors of any disease in South Africa. However, female *Simulium damnosum* complex transmit the filarial nematode *Onchocerca volvulus* in Central and West Africa, which causes onchocercosis or "river blindness" (Crosskey, 1990; Krueger, 2006; Palmer, 1997). In Uganda, it was estimated that 1.8 million people are at risk of being infected by this disease and about 1.36 million people are already infected with this disease (Lakwo *et al*., 2006). Although this vector can be found in South Africa, the pathogen is not yet present. According to Palmer (1997), *S. damnosum* complex was collected for the first time in the Berg and Olifants Rivers in 1995, whereas in the past, it was absent from the south-western Cape. Palmer (1997) also stated that "the increasing movement of people in southern Africa following the liberalisation of South Africa may well lead to the unwanted arrival of *O. volvulus* in South Africa".

Furthermore, female blackflies are ideally suited for mechanical transmition of disease (Palmer, 1997). In South Africa, blackflies have been implicated in spreading *Chlamydia* spp. (De Moor, 1982) and Rift Valley Fever virus (McIntosh *et al*., 1980). In animals, blackflies were also implicated in spreading bovine onchocercosis (Crosskey, 1990), the cytoplasmic polyhedrosis virus, the iridescent virus, vesicular stomatitis (Bridges *et al.,* 1997; Cupp *et al., 1992*), leucocytozoonosis (Kettle, 1992), avian trypanosomes (Crosskey, 1990), and myxomatosis (Williams and Williams, 1966).

1.2. Blackfly control in South Africa

The control of the larvae is the most effective as they are only found in fast running water, which makes it easy to locate the breeding sites (Palmer, 1997). In South Africa, control is primarily focused on the larval and pupal stages of blackflies. Myburg and Nevill (2003) reviewed blackfly control in South Africa.

1.2.1. DDT

Blackfly control in South Africa started in 1965 with DDT, which was applied to sections in the Vaal and Harts Rivers to attempt to control *S. chutteri* (Howell and Holmes, 1969). The trials were successful but there were negative outcomes. Fish and non-target invertebrates were also killed and there was excessive growth of benthic algae (Howell and Holmes, 1969). DDT provided a wide range of control at low cost but was harmful to the environment and this led to it being banned (Priest, 1992). The use of DDT was stopped for blackfly control in 1967 (Palmer *et al.*, 1996; Myburgh and Nevill, 2003).

2.2.1. Water flow manipulation

In the 1970s and 1980s, water-flow manipulation was used as a control tool (Myburgh and Nevill, 2003). Water-flow manipulation is when the water levels of the river are controlled by reducing the water level and then increasing it again (Palmer, 1997). When water levels are reduced, the blackfly pupae get exposed and desiccated (Fig. 1.9) (Howell *et al.*, 1981; Myburg and Nevill, 2003).

Fig. 1.9 Pupae that became desiccated during exposure.

In 1977, trials were done by Howell *at al.* (1981) at the Vaalharts irrigation weir and, in 1978, in the Orange River below the Van der Kloof and Buchuberg Dams. They tested the effects of water-flow manipulation on the numbers of Simuliidae. It was found that a 60-hour closure of dams in the Vaal and a 66-hour closure in the Orange River, could potentially be used for controlling the Simuliidae in these rivers. This type of control had limitations, i.e. the Simuliidae were only controlled for 30 km downstream from the Vaalharts weir, 370 km downstream from the Van der Kloof dam and 242 km downstream from Buchuberg Dam, with no control in the rest of the river (Howell *et al.*, 1981). This type of control did reduce the number of immature blackflies in the Vaal and Orange Rivers and it was estimated that the most advantageous time to manipulate water-flow is during July/August, as the greatest effect on larval populations can be found in the winter (Car, 1983).

However, the demand for water used in irrigation of crops that are grown along the Vaal and Orange Rivers and the water needed to generate power through hydroelectricity, make the reduction of water levels difficult. Considering all the factors, the use of water-flow manipulation as a means of control is difficult. De Moor and Car (1986) stated that this method is cost-efficient but it is impractical as it is limited by the availability of impoundments upstream of blackfly breeding sites.

3.2.1. Integrated water flow manipulation

Since water-flow manipulation on its own is not a sufficient control method (Myburgh and Nevill, 2003), the third method of blackfly control was Integrated Water Flow Manipulation. Data on the life-cycle, population dynamics and microhabitat preferences of the six most abundant *Simulium* species, and their aquatic invertebrate predators, were used to determine the best time to carry out a series of water-flow manipulations (De Moor, 1982). Therefore, water-flow manipulation is used for the primary control of blackflies followed by secondary control through predation (Myburgh and Nevill, 2003).

4.2.1. Larvicides

In South Africa, biological and chemical control of blackfly larvae can be achieved with the use of the larvicides, *Bacillus thuringienses* Barjac var. *israelensis* de Barjac (serotype H-14) (*B.t.i.*) and temephos ["Abate"-SA Cyanamid (Pty) Ltd]. *Bacillus thuringienses* Barjac var. *israelensis* is a naturally occurring bacterium that can be used for biological control of filter-feeding Diptera such as mosquitoes and blackflies and is highly specific (Myburgh and Nevill, 2003; Olejnicek, 1986; Priest, 1992). It is toxic to blackflies and mosquito larvae, and has minimal impact on non-target organisms (Molloy, 1992; Palmer 1993; Palmer and Palmer, 1995). *Bacillus thuringienses* Barjac var. *israelensis* needs to be ingested as it is a non-contact larvicide (Palmer, 1995). When B.t.i is ingested, proteins are released in the midgut. According to Gill *et al.* (1992), "…these toxins are activated by midgut proteases, the activated toxins interact with the larval midgut epithelium causing a disruption in the membrane integrity and ultimately leading to insect death". *Bacillus thuringienses* Barjac var. *israelensis* is currently used for the control of blackflies and mosquitoes in many parts of the world (Molloy and Struble, 1989; Becker and Rettich, 1994; Antwood *et al*., 1992; Ladle and Welton, 1996). In 1982, Car and De Moor (1984) conducted trials with *B.t.i* in the Vaal River and reported that it was successful in reducing the numbers of larval blackflies. However, laboratory studies revealed that control with *B.t.i.* could be problematic in polluted rivers (Car, 1984).

Temephos, on the other hand, is a sulphur-containing organophosphate widely used for the chemical control of mosquitoes and blackflies (Palmer, 1995; Palmer *et al*., 1996; Palmer, 1997; Myburgh and Nevill, 2003). The World Health Organisation's Onchocerciasis Control Programme first used Temephos for blackfly control in West Africa in 1974 (Palmer *et al.,* 1996). According to Myburgh and Nevill (2003), temephos gives the best results in rivers with silt particles in suspension. Temephos is absorbed into the silt particles and as the larva filters out the particles, it poisons itself. Temephos affects the nervous system by inhibiting the release of a certain enzyme (Palmer, 1995). Due to the mode of action and the low dosage rate (20% suspension concentrate), less temephos formulation is needed to produce the same effect as *B.t.i.* (Myburgh and Nevill, 2003).

In 1991, the ARC-OVI started extensive research projects along the lower Orange River to develop an environmentally-acceptable programme to control this blackfly species. A highly successful larval control programme was developed, using larvicides, and later implemented along 900 km of the Orange River (Palmer *et al.,* 2007). Both *B.t.i.* and temephos (organophosphate) proved to be effective against *S. chutteri* larvae and led to the establishment of an ongoing blackfly control programme along the Orange River (Palmer, 1997). The impact of these larvicides on other non-target organisms was also tested and it was found that they were safe to use in the Orange River (Palmer and Palmer, 1995). Larvicidal programmes for the control of blackflies were developed in both the Vaal and Orange Rivers but the program in the Vaal River was discontinued (Myburgh and Nevill, 2003; Myburgh, 2002).

5.2.1. Blackfly control problems

There are many problems with the current blackfly control in South Africa. As a biological control agent, *B.t.i* is successful but there are numerous factors that affect its efficacy. These factors include: aquatic vegetation, blackfly larval instar, pH, planktonic algae, pollution, water turbidity, water temperature, formulation, high organic content and free chlorine (Car, 1984; Olejnicek *et al.,* 1985; Molloy, 1990; Palmer, 1995; Palmer, 1997). Furthermore, according to Palmer (1995), *B.t.i.* does not "carry" as far as conventional larvicides, thus more applications are needed, which increases the cost of application. Another problem with *B.t.i* is that it has a limited shelf life (Palmer, 1995; Palmer, 1997). The shelf life is dependent on the formulation and although improvements have been made, it is advised that *B.t.i* is used as fresh as possible (Palmer, 1995).

According to Palmer (1995) the main disadvantage of using temephos is that it affects a wide variety of invertebrates. Palmer (1993) did a study of the short-term impacts of formulations of *B.t.i* and temephos and he also found that temephos significantly decreased the average number of taxa. The taxa that were most affected were blackflies *Simulium* spp., midges *Rheotanytarsus fuscus*, mayflies *Choroterpes elegans, Baetis glaucus*, caddisflies *Cheumatopsyche thomasseti* and cased caddisflies *Orthotrichia* spp. (Palmer, 1993). Palmer and Palmer (1995) indicated that the impact of repeated application of temephos in the Orange River is a significant reduction of the abundance of an estimated 25% of the invertebrate taxa in the stones-in-current biotope.

There are also other problems with larvicides. These products have to be delivered into the river simultaneously at predetermined points and there are logistical problems of getting the products to the right points along a river. Rivers are often inaccessible to vehicles, and helicopters are an expensive alternative. These products are not cheap, and each application would be costly (Bath, 2006 unpublished report, University of Pretoria). Insecticide resistance is also a problem due to overuse of larvicides. Resistance to insecticides develops when the same insecticide is applied frequently (Palmer, 1997). Resistance to *B.t.i* has developed in several pest species (Palmer, 1997).

1.2. Purpose of present study

According to Palmer *et al.* (2007), there were serious blackfly outbreaks in 2000 and 2001, which were attributed to higher than normal river levels and an alleged larval resistance to temephos. The current control in the Orange River is with *B.t.i.,* undertaken by the National Department of Agriculture as the Orange River Blackfly Control Programme. Blackfly annoyance is still high along the Orange River, however. Because of the problems, with the current control strategy in South Africa, futher research is needed to improve on blackfly control..

This led to the present investigation to assess the blackfly problem status along the Vaal and Orange Rivers as well as potential biological control agents that are environmentally friendly and cost effective. Surveys of the blackfly problem status and risk zones were, therefore, undertaken in 2006-2007. During these surveys, possible predators and other biological-control organisms were researched, together with water quality, silt levels, planktonic algae and other factors that could affect blackfly survival and may contribute to the biological control of the pest species. Studies also included the possible contribution of blackflies, predators and non-target organisms by tributaries of the Vaal and Orange Rivers. Therefore, the present study was launched to identify potential predators and other biological-control agents, that can play a rol in an Integrated Pest Management approach (IPM) to control the blackfly problem along the Vaal and Orange Rivers in South Africa.

1. SURVEY OF BLACKFLY ANNOYANCE LEVELS AND BREEDING SITES

1.2. Blackfly annoyances levels

In 1979, G.J. Begemann (unpublished data), of the Onderstepoort Veterinary Research Institute, first evaluated the extent of the blackfly problem along the Orange River with a single survey of questionnaires to farmers. In 1982, M. Car (unpublished data) also conducted a limited survey of follow-up questionnaires. In 1983, Jordaan and Van Ark (1990) surveyed blackfly annoyance of livestock along the Orange River extensively. Palmer (1997) continued with a smaller survey up to 1997. Between 1997 and 2002 E. Myburgh (unpublished data, 2002) continued to survey the annoyance of blackfies along the Orange River. In 1997 the survey was extended to include the Vaal River. The results of a limited survey carried out in 2006-2007 for the Orange and Vaal Rivers will be discussed.

Questionnaires were presented to livestock farmers along the Vaal and Orange Rivers to elucidate what the public views were concerning blackfly annoyance. The aims of the questionnaires were to determine, amongst others, the annoyance levels of the blackflies at various farms, what time of year the annoyance levels were at their peak, the distance from the river where problems may occur, the use of products against blackfly attacks, and also the number of farmers interested in participating in or being involved with blackfly research and control. An example of the questionnaire is given in Appendix 1.

As Jordaan and Van Ark (1990) also indicated, the aim of the questionnaires was to show pronounced general trends. Not too much value should be attached to specific numbers or frequencies and it should be emphasized that the information should not be regarded as objective.

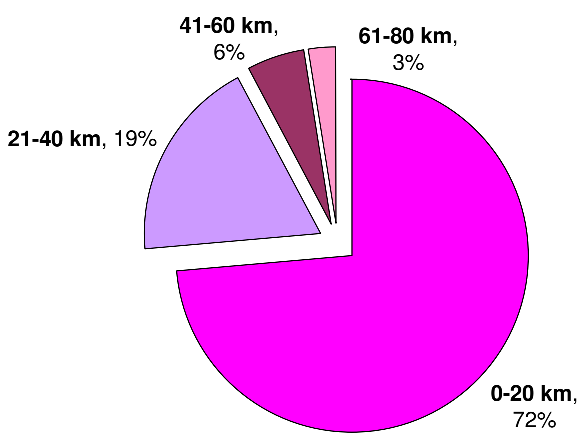
Fifty-one livestock farmers in different areas along the Orange River (see Fig. 2.11) were contacted telephonically. Farmers Unions (North West, Northern Cape and Free State) provided the necessary contact details. Thirty-nine (76%) of the farmers were willing to participate.

Sixty-two livestock farmers in different areas along the Vaal River (see Fig. 2.11) were contacted telephonically of which 52 (80%) were willing to participate in the telephonic questionnaires.

1.2.1. Distance from river

Farms were categorized into different zones according to distance from the rivers i.e.; 0-20 km, 21-40 km, 41-60 km and 61-80 km. Limited numbers of farmers were contacted further away from the river, therefore emphasis was placed on farmers closer to the river (0-20 km). On the Orange River, 72% of the farmers were located in the 0-20 km distance zone (Fig 2.1 A). On the Vaal River, 84% of the farmers were located in the 0-20 km distance zone (Fig 2.1 B).

A



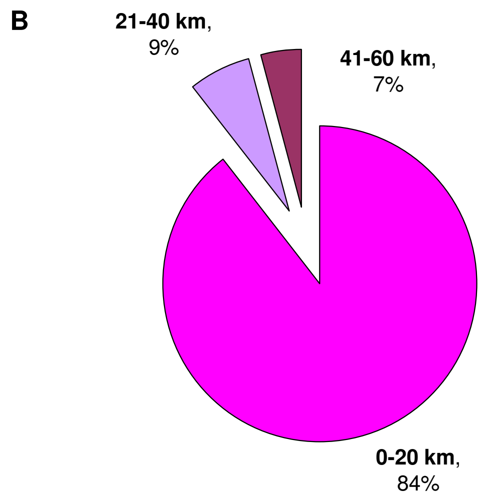


Fig. 2.1 Farmers contacted in the different distance zones in the (A) Orange and (B) Vaal Rivers.

2.2.1. Extent of blackfly problem and annoyance levels

Fig. 2.2 and 2.3 show the blackfly problem perception and rating scores, respectively, of the farmers contacted along the Orange and Vaal Rivers. All 39 farmers along the Orange River stated that they experienced a blackfly problem from time to time. A severe (9 -10) rating was allocated by 52% of the farmers, while none of the farmers allocated a non-severe rating (1-3) (Fig 2.4 A).

Along the Vaal River, 79% of the 52 farmers contacted stated that they experienced a blackfly problem from time to time, of which only 24% of the farmers gave the problem a severe rating of 9-10, while only 5% allocated a non-severe rating of 1 (Fig 2.4 B). Twenty-one percent indicated that they experienced no problem.

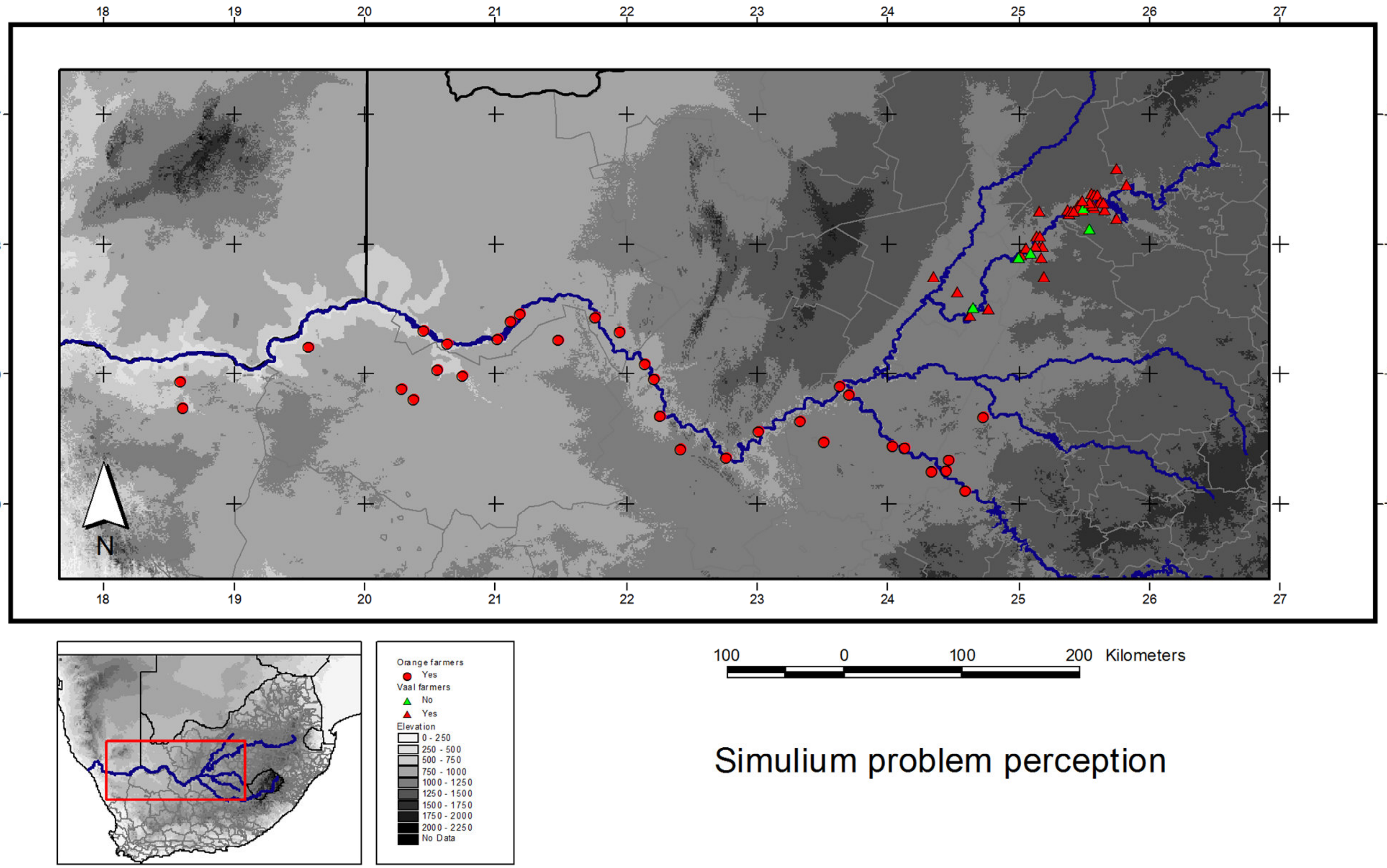


Fig. 2.2 Map showing distribution of farmers contacted and their problem perception along the Vaal and Orange Rivers. (Map produced by Guy Hendrickx, Avia GIS, 2007).

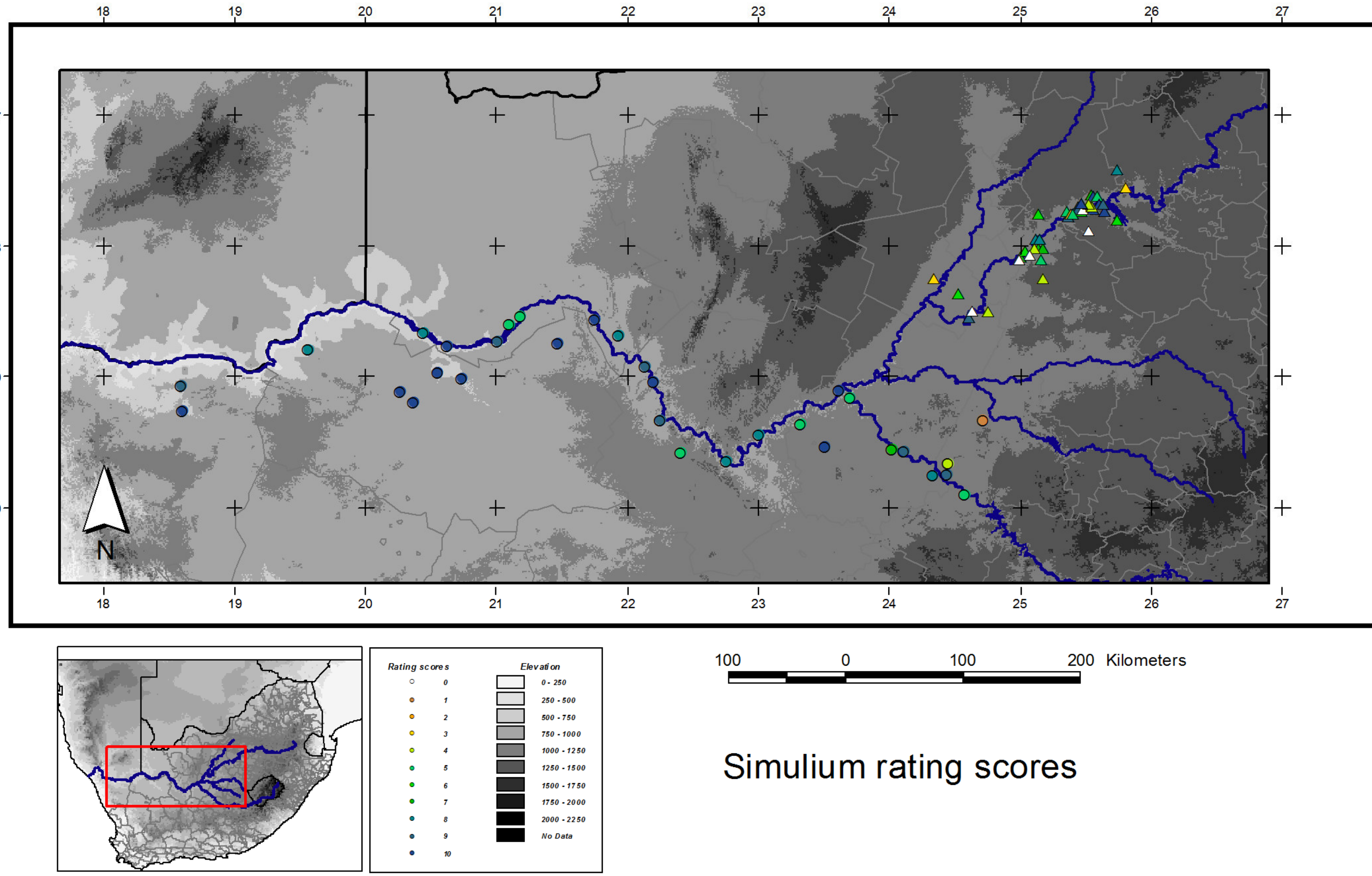


Fig. 2.3 Map showing distribution of farmers' rating scores along the Vaal and Orange Rivers. (Map produced by Guy Hendrickx, Avia GIS, 2007).

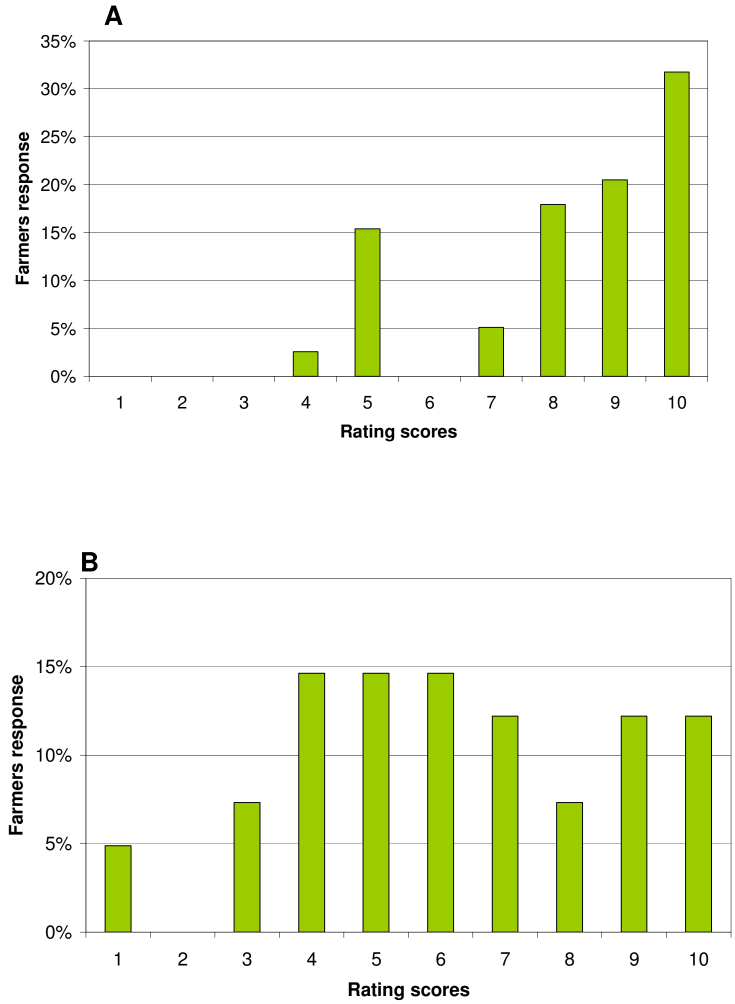


Fig. 2.4 Percentage of farmers that indicated the annoyance levels along the (A) Orange and (B) Vaal Rivers.

The farmers along both the Orange and Vaal Rivers indicated that the annoyance levels were the highest in the summer months October-March (Fig. 2.5 A, B) and the annoyance levels increased significantly after the first summer rains. Along both rivers the annoyance peaked in November-February. Thereafter the annoyance started to decrease gradually and was lowest in the winter months June-August.

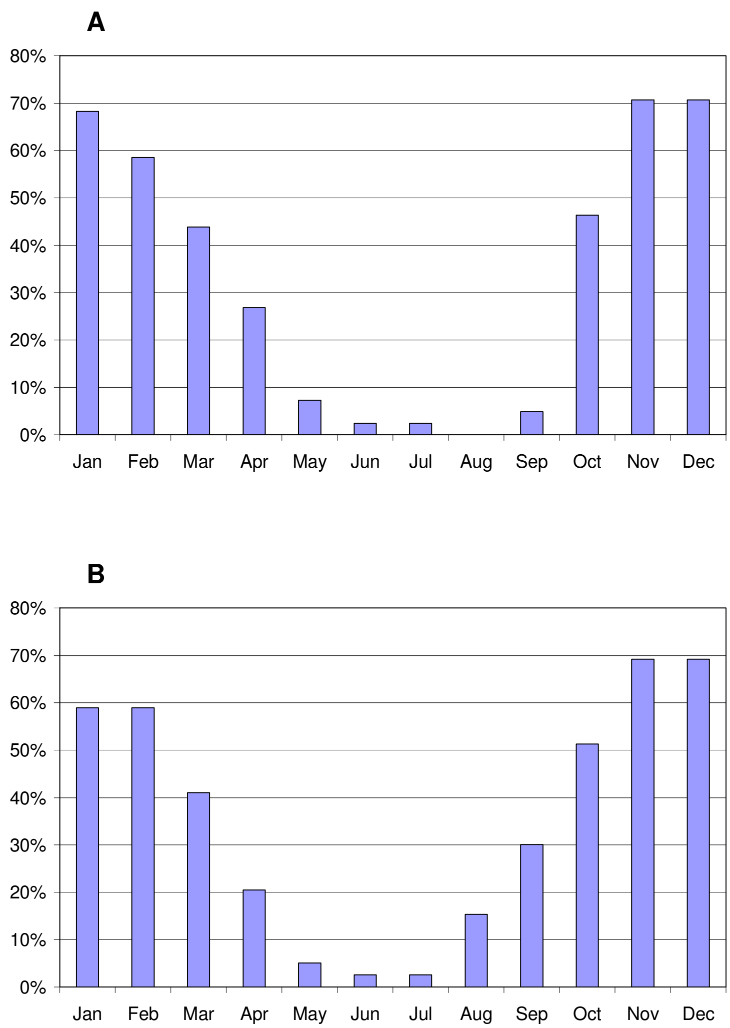
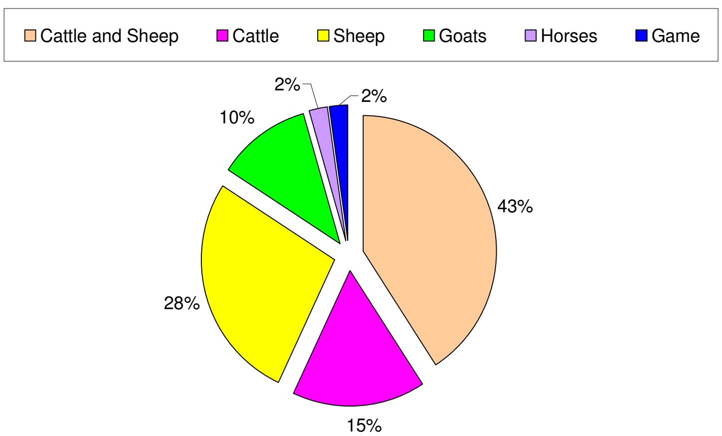


Fig. 2.5 Percentage of farmers who indicated highest blackfly annoyance for a specific month along the (A) Orange and (B) Vaal Rivers

3.2.1. Livestock and farming practices

The most common animals farmed with along the Orange River are cattle (58%) and sheep (71%) (Fig. 2.6 A). However, there are also some farmers that have horses as well as game and these animals are also hosts to blackflies. The largest numbers (68%) of these animals are kept in the open field most of the time (Fig 2.6 B). Some farmers have the facilities to keep the animals in the field and then to move them to feedlots as the need arises. Only 9% of farmers kept their animals permanently in feedlots.

A



B

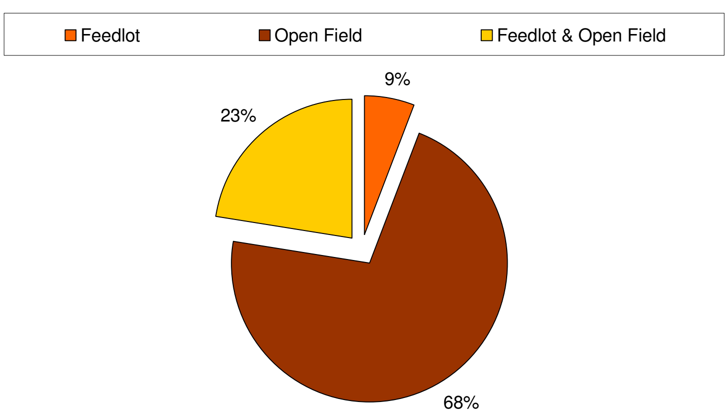
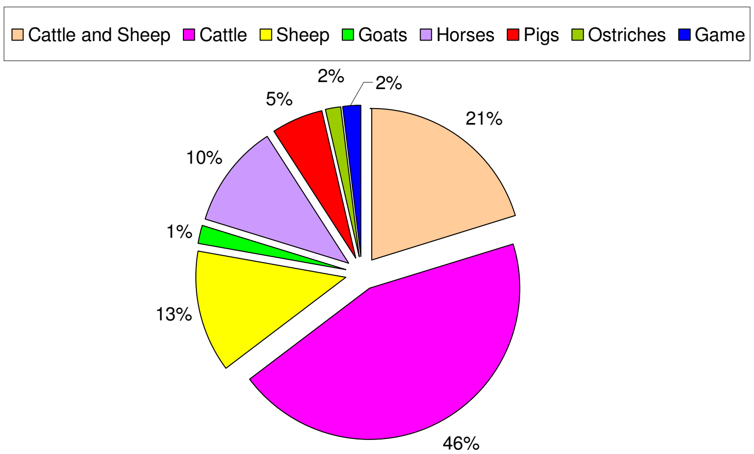


Fig. 2.6 Livestock farmed with (A) and where they are kept (B) along the Orange River.

As along the Orange River, the most common animals farmed with along the Vaal River are cattle (67%) and sheep (34%) (Fig. 2.7 A). However, there are also some farmers that have goats, pigs, ostriches, horses as well as game animals, which are also hosts to blackflies. The largest number of these animals are kept in the open field (58%) most of the time, whereas only 17% of farmers kept their animals permanently in feedlots (Fig. 2.7 B).

A



B

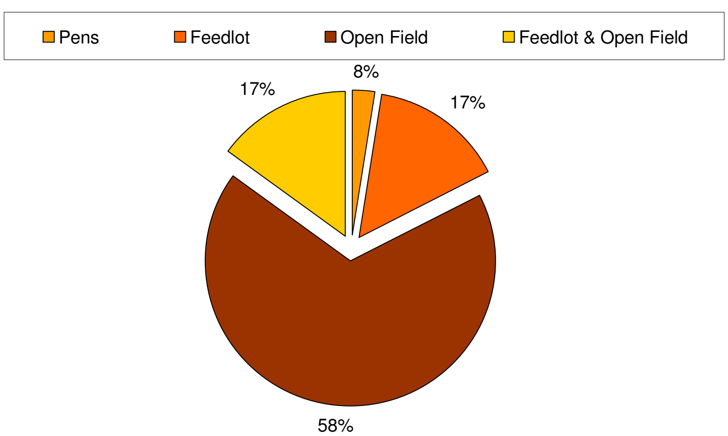


Fig. 2.7 Livestock farmed with (A) and where they are kept (B) along the Vaal River.

4.2.1. Products used against blackfly attacks

The farmers were asked whether they were aware of any registered products that could be used to protect livestock against blackfly attack. They were also asked if they have used any products for protection of their livestock and, if yes, whether they were satisfied or not and why not. Of the farmers along the Orange River, 43% indicated that they were aware of any products that can be used to protect livestock against blackfly attacks. Of these, 40% indicated that they used products (Fig 2.8A). Dazzel® and Parisite® were the two most frequently used products, as 53% and 20% of these farmers used these two products, respectively, on a regular basis. Only 27% of the farmers were satisfied with the results obtained from the use of various products (Fig 2.8 A). The rest of these farmers (73%) were not satisfied and stated that the various products were too expensive and only worked for a limited time. They regarded the application of the products as labour-intensive as the animals needed to be taken out of the field and then treated. The overall consensus was that it was not sufficient to only treat the animals, but that blackflies as a pest needed to be controlled before they reached the animals.

Along the Vaal River, 43% of farmers indicated that they were aware of any products that can be used to protect livestock against blackfly attack. Of these, 49% indicated that they used products (Fig 2.8 B). Clout® and Deadline® were the two most frequently used products, as 20% of the farmers used these two products on a regular basis. Fifty-five percent of the farmers were satisfied with the results obtained from the use of a variety of products, while the rest of the farmers (20%) were not satisfied, 5% were only partly satisfied and 20% did not know whether these products worked for blackfly annoyance (Fig 2.8 B). Therefore, contrary to the responses of farmers on the Orange River, the majority of farmers along the Vaal River stated that they were satisfied.

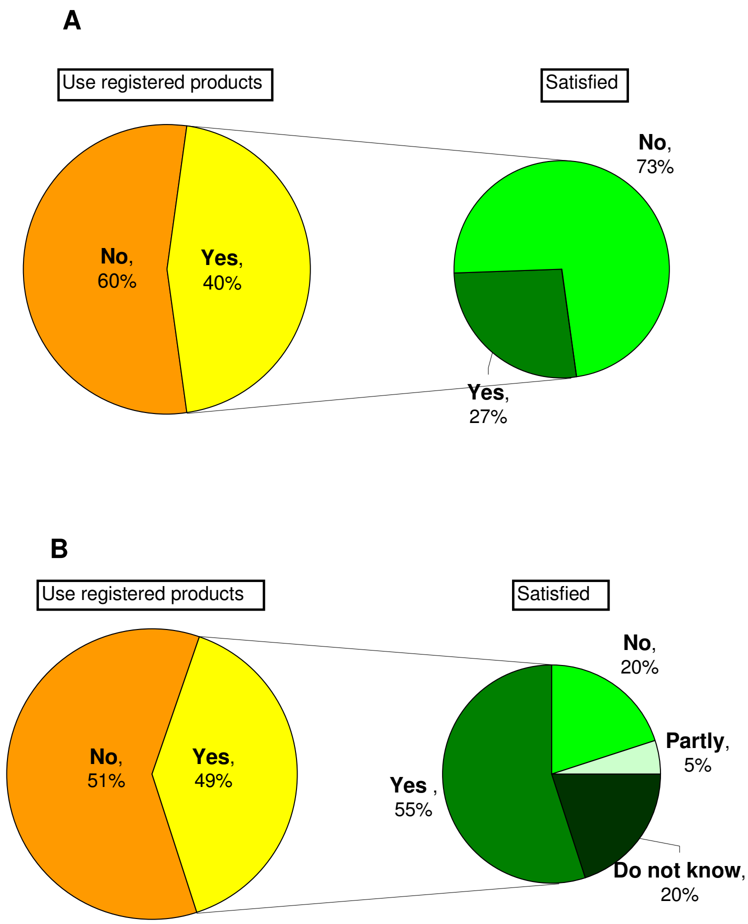


Fig. 2.8 Farmers' views on the use of products to protect livestock against blackfly attacks along the (A) Orange and (B) Vaal Rivers.

5.2.1. Farmers' involvement

The distribution of farmers along the Orange and Vaal Rivers that were willing to become involved in the blackfly control research conducted by ARC-OVI, aimed at bringing control solutions for farming communities, are indicated in Fig 2.9. Of the thirty-nine farmers contacted along the Orange River, 92% (Fig 2.10 A) stated that they would like to be part of the blackfly control research

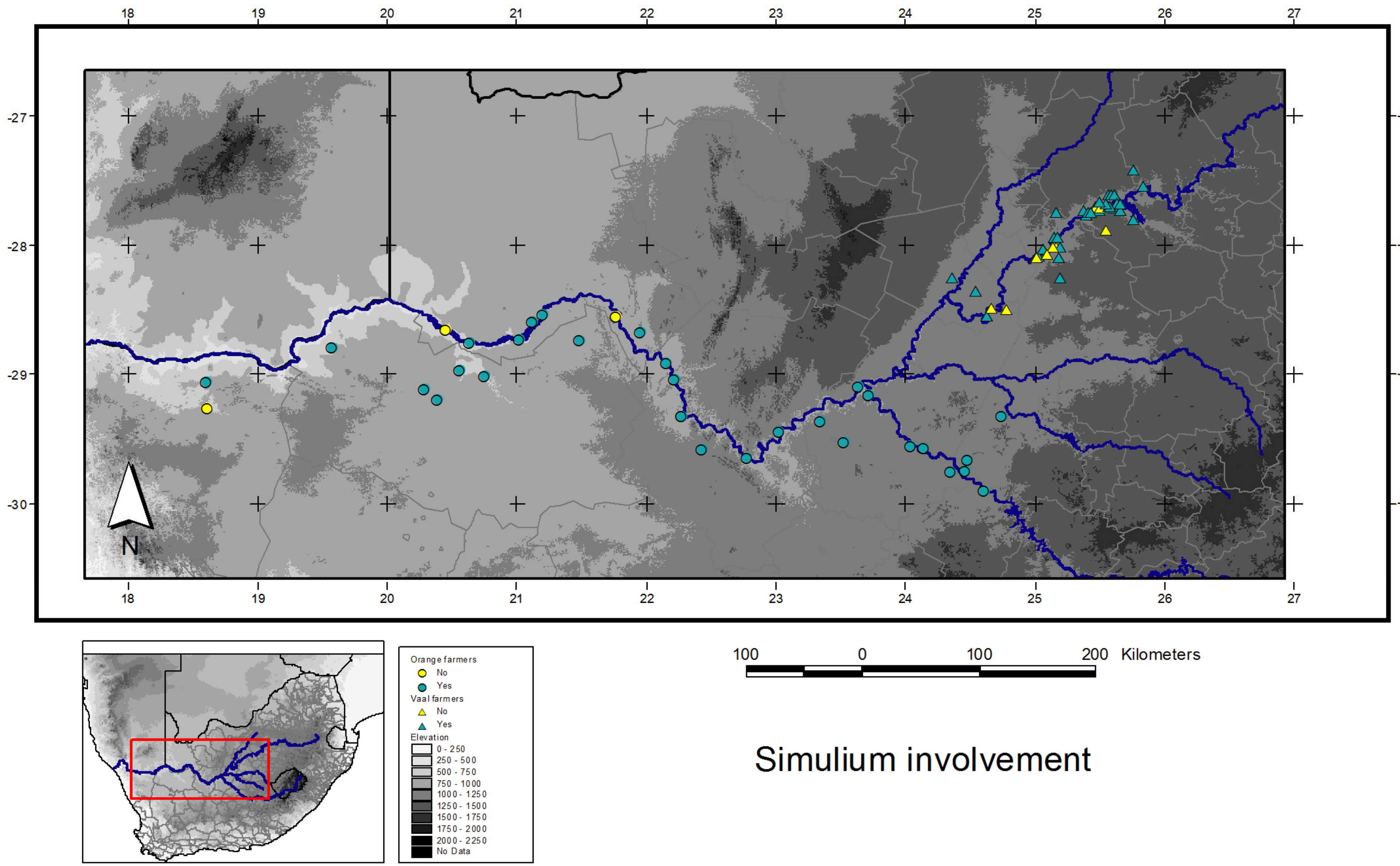


Fig. 2.9 Map showing distribution of farmers' involvement along the Vaal and Orange Rivers. (Map produced by Guy Hendrickx, Avia GIS, 2007).

and of the 52 farmers contacted along the Vaal River 90% (Fig 2.10 B) stated that they would also like to be part of the blackfly control research.

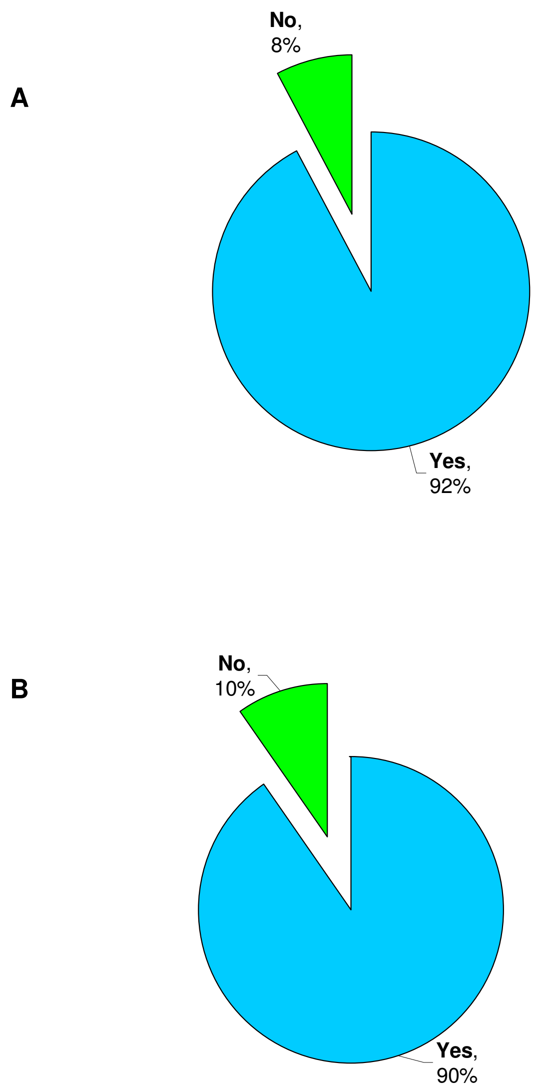


Fig. 2.10 Farmers' willingness to become involved in the Blackfly Control Research along the (A) Orange and (B) Vaal Rivers.

1.2. Study sites

A number of rapids were visited along the Orange and Vaal Rivers in order to locate the specific blackfly breeding sites or high-risk areas. Various breeding sites were identified for repeared surveying during follow-up visits (Fig 2.11). These breeding sites were surveyed between September 2006 and September 2007. Tributaries of the Vaal River, namely the Riet, Modder and Harts Rivers, were also surveyed to determine their possible contribution of blackflies, predators and non-target organisms.

1.2.1. Orange River

The study sites along the Orange River (running from east to west) are situated from the upper part of the river at Van der Kloof Dam to the lower part of the river at Onseepkans (see Fig 1.7). Thirteen sites (Fig. 2.11) were chosen. A description of each of the study sites, including their latitude, longitude, elevation and marginal vegetation, is given in Table 2.1, with site photographs and satellite views given in Appendix 2.

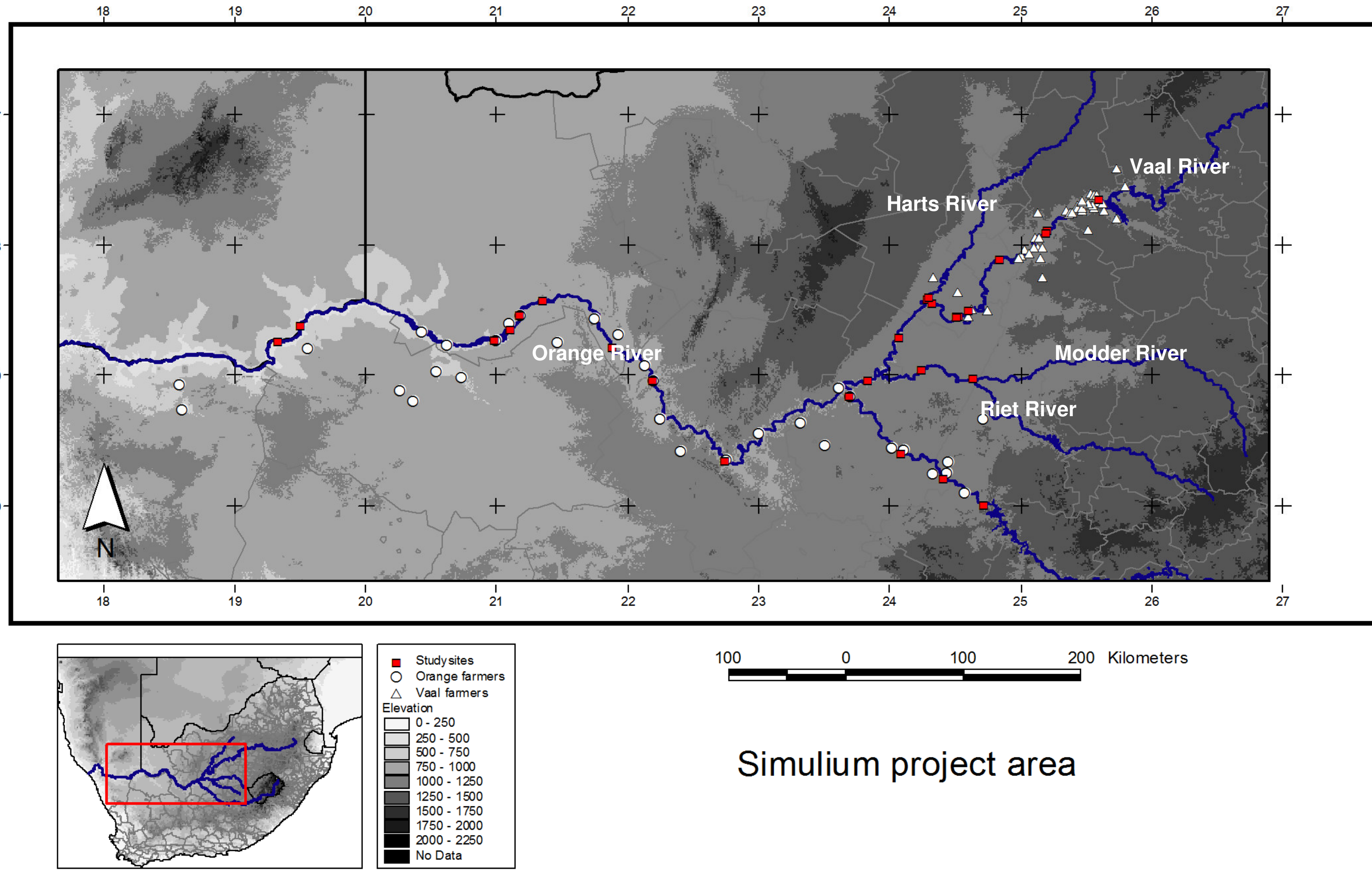


Fig. 2.11 Study sites in the Vaal, Riet, Modder, Harts and Orange as well as the livestock farmers that were contacted through questionnaires (Map produced by Guy Hendrickx, Avia GIS, 2007).

Table 2.1: Study sites along the Orange River

Site Name	Lat. (S)	Long. (E)	Elev. (m)	Distance from V. D. Kloof dam (km)	Breeding Site Description	Marginal Vegetation	App. Ref.
Van der Kloof	29°59’33”	24°43’26”	1100	0.8	Solid rock beds, high water level fluctuations, sampling in low water-flow areas.	*Cyperus marginatus, Phragmites australis, Schoenoplectus paludicola*	2.1
Fluitjieskraal Bridge	29°47’25”	24°24’31”	1076	48.2	Grooved rock slabs, solid rock bed, 30 meters from the bridge, large quantities of grey slime algae, small amounts of loose sampling stones, island with reeds and two trees.	*C. marginatus*	2.2
Hopetown	29°36’01”	24°05’17”	1046	98.2	Continuous wide flat open rock beds, small medium rocks, rapid system approximately 100 meters long, rapid water-flow.	*Spirodela* spp., *Berula erecta, Nasturtium officinale, Veronica anagallis aquatica, Juncus lomatophullus, C. marginatus, P. australis*	2.3
Marksdrift	29°09’45”	23°41’39”	970	174.6	Large rapid, located under a bridge, downstream from a gauging station, large rocks, rapid water-flow.	*C. marginatus, P. australis.*	2.4
Prieska	29°39’26”	22°44’39”	916	355.1	Downstream of a bridge, concrete slabs and rubble from the old bridge, medium-sized natural rocks, rapid water-flow, steep slope.	*P. australis*	2.5
Buchuberg	29°02’36”	22°11’53”	911	468.3	Downstream from Buchuberg dam, continuous pebble bed, water-flow fast on an open area, reed bed on the side of the river, reed island.	*P. australis*, *C. marginatus*	2.6
Sishen Bridge	28°47’14”	21°52’45”	839	530.1	Large rapid, located under railway bridge, rock bed facing upstream, slope of approximately one meter, reed beds on the sides of the river, drifting vegetation.	*C. marginatus, P. australis*	2.7
Strausbury	28°25’52”	21°21’17”	796	625.5	Located in a branch of the main river, series of large boulders, small pebbles and broken rock beds, rapid water-flow, reed island on both sides of the river.	*C. marginatus*, *P. australis* and *N. officinale*	2.8
Ses Bridge	28°32’37”	21°10’39”	769	653.8	Large granite boulders, rapid water-flow, island of reeds and rocks.	*P. australis, C. marginatus*	2.9
Kanoneiland	28°38’53”	21°06’08”	761	666.5	Downstream from gauging wall, upstream from a bridge, rapid water-flow over large granite rock slabs, small sampling rocks, reed beds, island of reeds and rocks.	*C. marginatus*, *P. australis*	2.10
Keimoes	28°43’40”	20°59’07”	714	683.8	Located at bridge, reed island that cuts into the stream, solid granite slabs with smooth surfaces and a steep slope, small loose rocks, rapid water-flow, reed beds on side of the river.	*C. marginatus*, *P. australis*	2.11
Raap en Skraap	28°37’37”	19°30’17”	392	870.0	Series of boulder ridges, rapid water-flow, series of reed and stone Islands, reed beds on either side of the river.	*P. australis*	2.12
Onseepkans	28°44’33”	19°20’04”	363	895.2	Rapid and pool system, flat flowing slope, solid rock beds, reed and rock islands.	*P. australis*	2.13

2.2.1. Vaal River

The study sites along the Vaal River are situated from the Bloemhof Dam up to where the Vaal joins the Orange River (see Fig 1.7). Twelve study sites (Fig. 2.11) were chosen. Each of the study sites is described briefly in Table 2.2, and photographs and satellite views given in Appendix 3.

3.2.1. Tributaries of the Vaal and Orange Rivers

Tributaries of the Vaal and Orange Rivers were investigated to determine their contribution of blackflies, predators and non-target organisms. Four tributary rivers of the Orange River were identified, namely the Vaal, Hartbees, Sout and Molopo rivers. However, three of these rivers only flow in wet years and do not make a significant contribution to the fauna and flora of the Orange River. The only river that contributes significantly to the fauna and flora of the Orange River is the Vaal River.

In the Vaal River two tributaries were monitored (Fig. 2.11), namely the Harts and Riet Rivers. The Modder River, a tributary of the Riet River, was also surveyed at a single site. Each of the sites is described in Table 2.3, with site photographs and satellite views given in Appendix 4.

Table 2.2: Study sites in the Vaal River.

Site Name	Lat. (S)	Long. (E)	Elev. (m)	Distance from Bloemhof (km)	Breeding Site Discription	Marginal Vegetation	App. Ref.
Bloemhof	27°39'15"	25°35'44"	1205	3.0	Downstream from gauging station, upstream from bridge, water is flat leading towards the rapid, small rocks, willow trees extend to the water's edge.	*Cyperus marginatus*	**3.1**
Nkolo Spa	27°53'11"	25°12'29"	1194	57.2	Located in branch of the main river, long rapid system, small to medium-sized rocks, rocks exposed, grass and weed vegetation island, wind protected, long reeds on river bed.	*Phragmites australis, C. marginatus, Cyperus eragrostis*	**3.2**
Christiana	27°54'21"	25°11'40"	1189	60.5	Continuous rapid, granite rock bed of medium-sized rocks, small rock and vegetation island, reeds on both banks of the river.	*P. australis, C. marginatus*	**3.2**
Warrenton	28°06'21"	24°50'35"	1161	111.7	Located at low water bridge, continuous rapid, small and medium-sized rocks, pebble beds, series of rock and vegetation islands, river in small channels.	*P. australis, C. marginatus, Juncus lomatophyllus, Berula erecta*	**3.3**
River Mead	28°29'58"	24°36'16"	1094	188.9	Located at gauging weir, solid rock bed, no small rocks, algae in high quantities, disturbed by mining activity in July 2006. Sampling continued on opposite bank of river.	*P. australis, Ceratophyllum demersum, Potamogeton pectinatus, C. marginatus*	**3.4**
Barkly West (Rietgat)	28°32'54"	24°31'43"	1086	215.7	Located at gauging weir, leak in weir's wall, pebble bed, steep gradient, well protected as it is somewhat isolated from the main river, reeds on either side of this small stream.	*Cyperus dives, V. a. aquatica, P. pectinatus, C. marginatus*	**3.5**
Rekaofela Resort	28°33'15"	24°30'25"	1081	217.7	Long natural pebble bed, continuous drop, rapid steps, exposed rock, vegetation islands.	*P. australis*	**3.5**
Delportshoop	28°25'02"	24°17'25"	1003	252.3	Located downstream of a gauging weir and a bridge, pebble bed, extends from below the bridge, with slope, vegetation island.	*P. australis, C. marginatus, P. pectinatus*	**3.6**
Sydney on Vaal	28°27'01"	24°19'33"	1003	257.6	Located at a tailing bridge, pebble bed, mining activity, a delta made of pebble and vegetation islands.	*C. demersum, P. pectinatus, C. marginatus, J. lomatophyllus*	**3.7**
Schmidtsdrif	28°42'44"	24°04'21"	990	307.7	Located downstream of the gauging weir, shallow part of the river with the maximum depth being approximately 0.7m, wide variety of algae present, open flat rock bed of small to medium rocks.	*P. australis, C. marginatus, P. pectinatus, C. demersum*	**3.8**
Douglas	29°02'40"	23°50'09"	988	374.3	Located downstream of a small dam, 22.83 km upstream from where the Vaal meets the Orange River, large water-flow fluctuations, solid rock beds with loose scattered rocks, extensive flat water areas, areas with fair gradients, reed beds present.	*P. australis, C. demersum, C. marginatus*	**3.9**

Table 2.3: Study sites on the largest tributaries to the Vaal River

Site Name	Latitude (S)	Longitude (E)	Elevation (m)	Breeding Site Discription	Marginal Vegetation	App. Ref.
Harts	28°24'30"	24°17'50"	1109	Located downstream from a small bridge, pebble bed with rapid steps, fair gradient, larger exposed rocks, pools of water up and downstream from rapid, large quantities of salt, 1.65 km upstream from where it joins to the Vaal River.	*Potamogeton pectinatus*	**4.1**
Riet	28°57'60"	24°14'43"	1020	Located at a dam wall, small zone of 10 m wide, solid rock face steps, small stones, large quantities of grey and brown slime algae, 56.38 km upstream from where it joins the Vaal River.	*P. australis, P. pectinatus.*	**4.2**
Modder	29°01'67"	24°38'28"	1003	Located downstream of the gauging station and a bridge, frequent of water-flow fluctuations, flat pebble bed, reeds on both the river banks, 2.76 km upstream from where it joins the Riet River.	*P. australis, C. marginatus.*	**4.3**

1. BLACKFLY SPECIES AND ABUNDANCE, RIVER CONDITIONS AND ALGAE COMPOSITION

1.2. Introduction

In South Africa, periodic outbreaks of blackflies have been reported in both the Vaal and Orange Rivers (Palmer, 1997). These blackfly outbreaks have a major effect on the livestock and labour-intensive farming systems in South Africa (Palmer *et al*., 1996; Palmer, 1997). Blackfly control is thus important in South Africa. Presently, control in the Orange River is done by the use of *Bacillus thuringienses* var. *israelensis* (Myburgh and Nevill, 2003) and no control is done in the Vaal River.

Knowing blackfly abundance is important in the planning of a control system. Population size of blackflies is determined by environmental conditions such as air temperature, evaporation, wind, water flow, water temperature and total suspended solids (Palmer, 1997). Therefore, continuous measurements of these conditions need to be recorded.

Identification of South African blackflies is also important and difficult as identification tools are outdated and widely dispersed in the literature (Palmer, 1997). On the Orange River, *Simulium chutteri* is regarded as the most important pest species (Palmer, 1997). *Simulium chutteri* as well as *S. damnosum* s.l. and *S. adersi* can be seen as problem species in the Vaal River.

To determine the present state of the blackfly problem along the Orange and Vaal Rivers, studies were undertaken to monitor the abundance of blackflies in these rivers. Tributaries of the Vaal River, namely the Riet and Harts Rivers, were also monitored to determine their possible contribution of blackflies, predators and non-target organisms to the Vaal River. The river conditions were recorded for this time period and related to the abundance of the blackflies. The blackfly species were also identified at selected sites in order to confirm important pest species along the Vaal and Orange Rivers. Samples of algae species at each site were identified also to be related to blackfly abundance so as to determine if any algae may have a negative effect on blackfly populations.

1.2. Materials and methods

1.2.1. Blackfly sampling and identification

Adult blackflies were collected during the various seasons at selected sites along the Orange and Vaal Rivers from September 2006 until September 2007. Collections were made using hand nets at each of the selected breeding sites described in Chapter 2. Adults were preserved in 70 % ethanol in order to identify the species composition at certain breeding sites.

The populations of immature stages were monitored using the 10-point visual ranking system for flat surfaces (Fig. 3.1 A & B) and cylindrical surfaces (Fig. 3.2 A & B) developed by Palmer (1994). In Table 3.1 the classes used in the 10-point visual ranking system can be seen, which correspond to the numbers of blackflies on the selected surfaces (Palmer, 1994). Larvae and pupae were collected from six randomly selected attachment sites, i.e. three flat surfaces (stones) and three cylindrical surfaces (vegetation).

Pupae were identified (Palmer, 1991) from six breeding sites, i.e. two sites in the Vaal River, two in the Orange River and one site in each of the tributaries, i.e. the Riet and Harts Rivers.

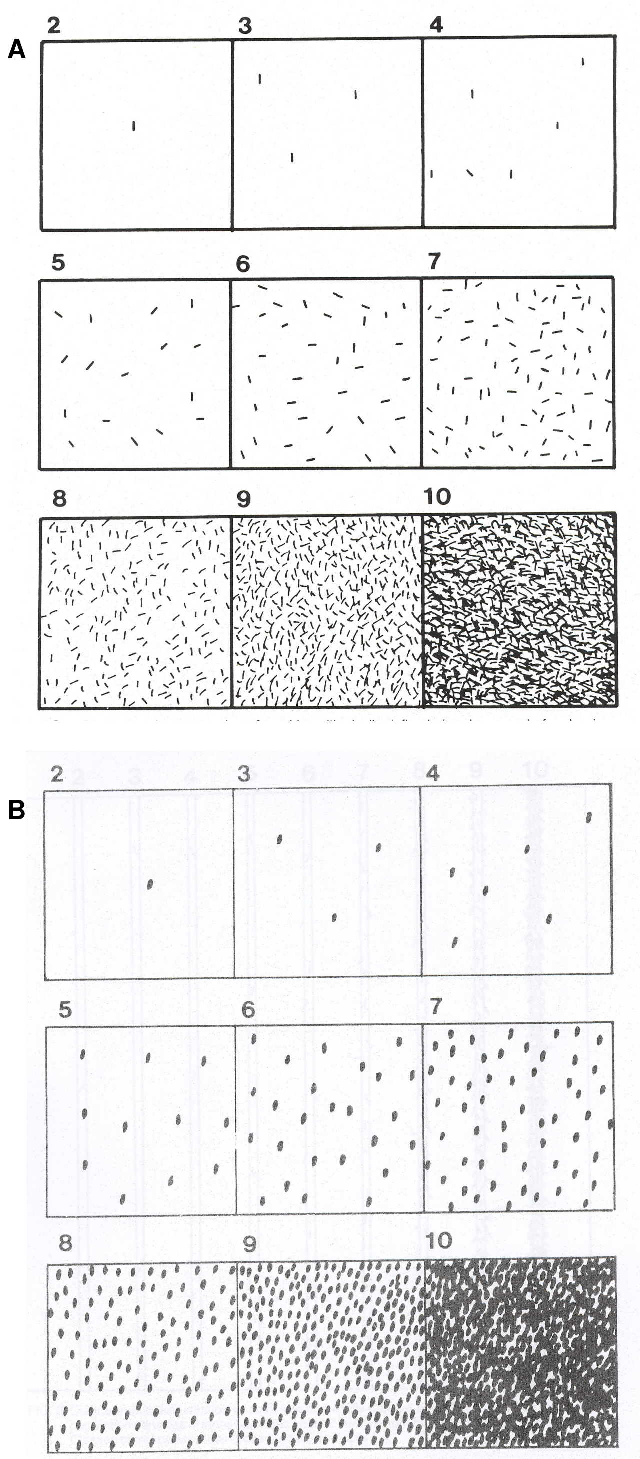


Fig. 3.1 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of larval blackflies about 2 mm (A) and pupal blackflies about 2-3 mm (B) in length found on flat substrates, such as stones or leaves (Palmer, 1994) (Reduced scale; the blocks should be 4x4 cm).

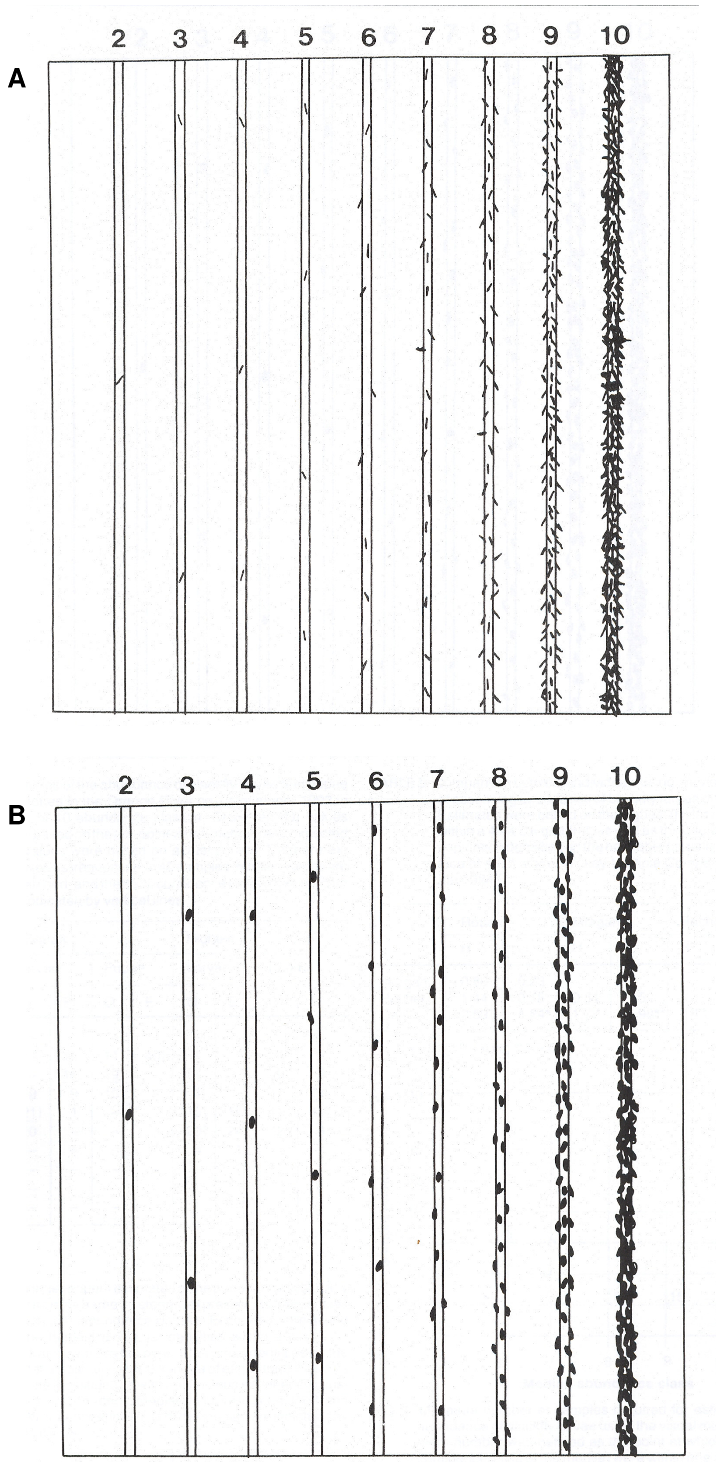


Fig. 3.2 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of larval blackflies about 2 mm (A) and pupal blackflies about 2-3 mm (B) in length found on cylindrical substrates (Palmer, 1994) (Reduced scale; the colums should be 13cm long).

Table 3.1: The number of larvae and pupae per 16cm² in each of 10 abundance classes used for estimating the abundance of immature blackflies. Ranges for the classes are given in brackets (from Palmer, 1994).

Class	Larvae	Pupae
1	0	0
2	1 (1-2)	1 (1-2)
3	3 (3-4)	3 (3-4)
4	6 (5-9)	6 (5-8)
5	16 (10-22)	11 (9-15)
6	36 (23-58)	25 (16-35)
7	88 (59-120)	55 (36-80)
8	202 (121-310)	120 (81-180)
9	500 (311-800)	280 (181-400)
10	1 050 (> 800)	600 (> 400)

2.2.1. River conditions

Spot measurements of water temperature were taken at each site when blackflies were collected. Hourly measurements of water flow and water level were taken at selected sites in all the rivers with electronic loggers provided by the Department of Water Affairs and Forestry. These data were presented as mean daily measurements (App. 5 & 6). The selected sites for the Vaal River were Bloemhof, Warrenton, River Mead, Rietgat, Delportshoop and Schmidtsdrif and for the Orange River they were Van der Kloof, Marksdrift, Prieska, Buchuberg, Strausbury, Keimoes and Onseepkans. There was also one site in each of the Harts and Riet Rivers.

3.2.1. Algae collections and water turbidity

Water samples, preserved with formalin, were collected at all the sites during all the collection periods and sent to the Centre of Environmental Management at the University of the Free State. Samples were analysed for algae composition and water turbidity was measured.

4.2.1. Data analyses

Spot measurement data that were collected at the sites for river conditions, algae abundance, and blackfly abundance were analysed using the statistical software Gen Stat (2003) to determine whether data records differed between sites and seasons. Repeated measures ANOVA (*P* value < 0.05 was considered as significant) were applied to all the data. Where the data passed the normality test, standard (parametric) methods were used and the Tukey test was applied. In the case where the data did not pass the normality test, nonparametric methods were used and the Friedman test was applied. The abundance of blackfly species was also correlated with environmental conditions and algae abundance using a linear regression analysis. The Vaal, Riet, Modder and Harts Rivers were analysed as one unit and the Orange River on its own.

1.2. Results

1.2.1. Blackfly species

As indicated above, the pupae at selected sites were identified. These were at Christiana and Delportshoop in the Vaal River, Marksdrift and Ses Bridge in the Orange River and one site in each of the Harts and Riet Rivers. In the Vaal River, five species were identified, namely *S. chutteri*, *S. damnosum* s.l, *S. hargreavesi, S. adersi* and *S. alcocki* (Table 3.2) of which *S. adersi* was the most common species throughout the seasons..

Table 3.2: Pupae of various blackfly species found at selected sites in the Vaal, Orange, Harts and Riet Rivers.

Vaal River		**Christiana**		**Delportshoop**	
Season		**Stones**	**Vegetation**	**Stones**	**Vegetation**
Summer	*S. alcocki*	1			
	S. chutteri				1
	S. adersi		3	23	14
	S. damnosum s.l.		7	15	7
	S. hargreavesi			48	46
Autumn	*S. alcocki*	2			
	S. adersi			6	5
	S. damnosum s.l.				2
	S. hargreavesi				3
Winter	*S. alcocki*	1	5		
	S. chutteri	4	5		1
	S. adersi	1	6	4	25
	S. damnosum s.l.	2	23		7
	S. hargreavesi			12	6
Spring	*S. alcocki*	6			
	S. chutteri	8		6	
	S. adersi	2			2
	S. damnosum s.l.			1	14
	S. hargreavesi				2

Orange River		**Marks drift**		**Ses Bridge**	
Season		**Stones**	**Vegetation**	**Stones**	**Vegetation**
Summer	*S. chutteri*	2		2	
Autumn	*S. chutteri*	73	12		5
	S. adersi		19		
	S. damnosum s.l.		1		
Winter	*S. chutteri*	397	303	61	34
	S. alcocki				2
	S. gariepense	2			
Spring	*S. chutteri*	331	188	16	38
	S. adersi				2
	S. alcocki				34
	S. gariepense	12			

Tributaries		**Harts River**		**Riet River**	
Season		**Stones**	**Vegetation**	**Stones**	**Vegetation**
Summer	*S. adersi*		15		
Autumn	*S. adersi*	4	7		10
	S. damnosum s.l.			7	3
	S. hargreavesi			3	5
Winter	*S. adersi*		15		68
	S. damnosum s.l.				5
	S. hargreavesi			1	2
Spring	*S. adersi*				2
	S. damnosum s.l.			1	43

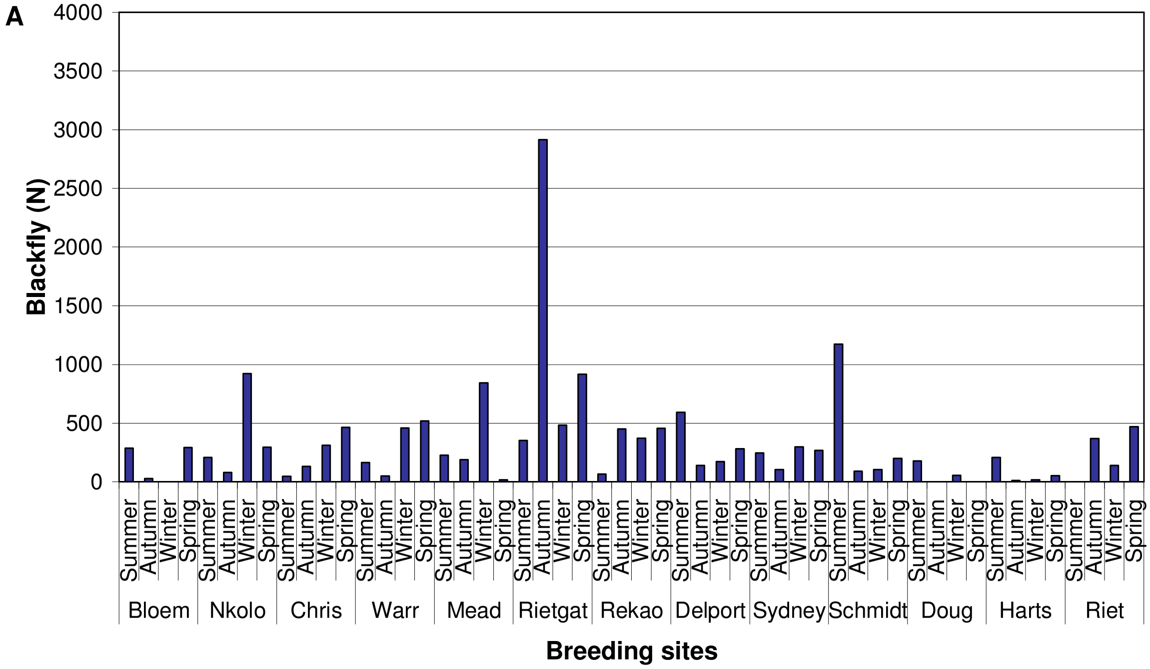
Furthermore, there were differences between the species found at the two sites in die Vaal River. *Simulium alcocki* was present at Christiana but absent at Delportshoop. The opposite was found for *S. hargreavesi. Simulium hargreavesi (35%)* was the most abundant species at Delportshoop and *S. damnosum* s.l. (39%) at Christiana. In the Vaal River the highest species diversity occurred in winter.

Five species, namely *S. chutteri*, *S. damnosum* s.l., *S. adersi, S. alcocki and S. gariepense,* were identified at the two sites in the Orange River. Of all pupae identified, 95% were *S. chutteri* (Table 3.2). *Simulium damnosum* s.l. and *S. gariepense* were present at Marksdrift only and *S. alcocki* at Ses Bridge only. The largest variety of species was found during spring. Only *S. gariepense* and *S. chutteri* were located on the stones; the rest of the species were only found on the vegetation. *Simulium adersi* was also the most abundant species in the Harts (100%) and Riet (53%) Rivers (Table 3.2).

2.2.1. Blackfly numbers

The numbers of blackflies sampled seasonally at the various sites along the Vaal and Orange Rivers are indicated in Fig. 3.3A & B. In the Vaal River, more than 1000 blackfly larvae and pupae were collected at two sites, namely Rietgat (Barkly West) and Schmidtsdrif. There were no significant differences in blackfly numbers between the sites *($P = 0.124$)*. There were also no significant differences in the blackfly numbers between the seasons *($P = 0.986$).*

For the Orange River, more than 1000 blackfly larvae and pupae were collected at seven sites. There were no significant differences between the number of blackfly larvae and pupae collected at the sites *($P = 0.193$)*. There were, however, high significant differences of blackfly numbers between the seasons *($P < 0.001$)*. The upper part of the Orange River had the highest larvae and pupae numbers.



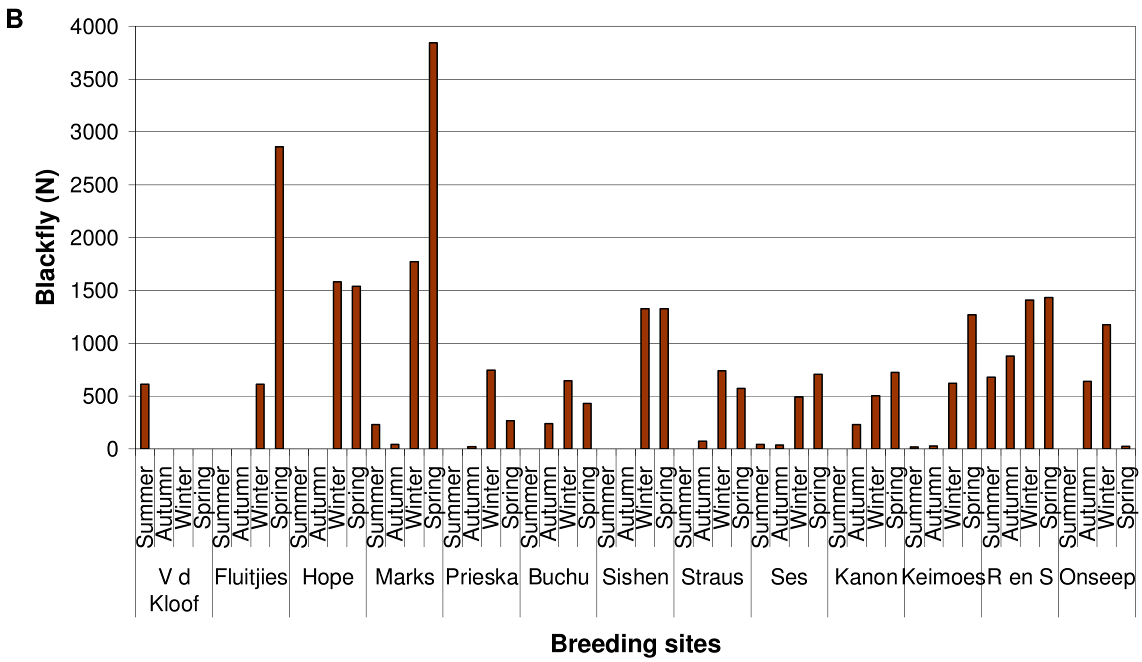


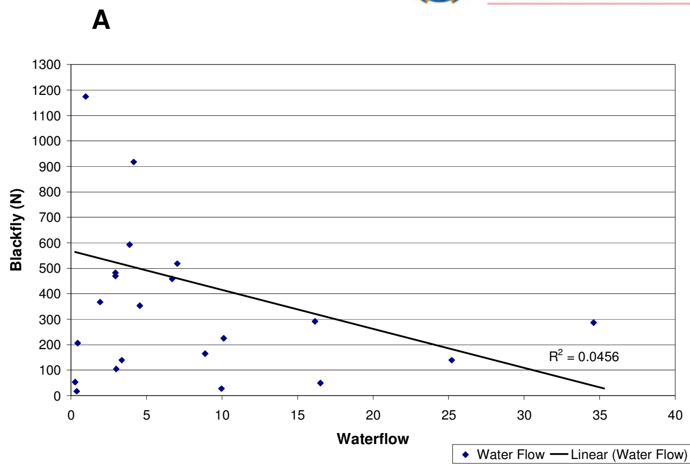
Fig. 3.3 Blackfly larvae and pupae numbers in the Vaal (A) and Orange (B) Rivers.

3.2.1. River conditions

Water flow

The monthly mean minimum and maximum flow for the Vaal, Harts, Riet, Modder and Orange Rivers are given in Appendices 5 and 6. At Port Arlington in the Vaal and Dooren Kuilen in the Orange River, large variations can be seen between the monthly minimum and maximum flow. The reason for this is that these sites are located below Bloemhof and Van der Kloof Dams, respectively. In the Orange River, a significant peak in water flow was seen in November 2006. This occurred when the Orange River had a high flow, which made sampling difficult. In the Vaal River, levels of high flow were experienced in June 2007. It is evident, therefore, that the water flow in the Vaal River differed from that of the Orange River.

In the Vaal River, there were high significant differences in the water flow between sites *($P < 0.001$.)* In the Orange River there were high significant differences between seasons *($P = 0.001$)*. The correlation between blackfly numbers and water flow was not significant in both the Vaal ($R^2 = 0.05$) and Orange ($R^2 = 0.21$) Rivers (Fig 3.4 A & B). However, for both rivers there was a slight negative correlation between blackfly adundance and water flow (Fig 3.4 A & B), thus the higher the waterflow, the lower the blackfly abundance.



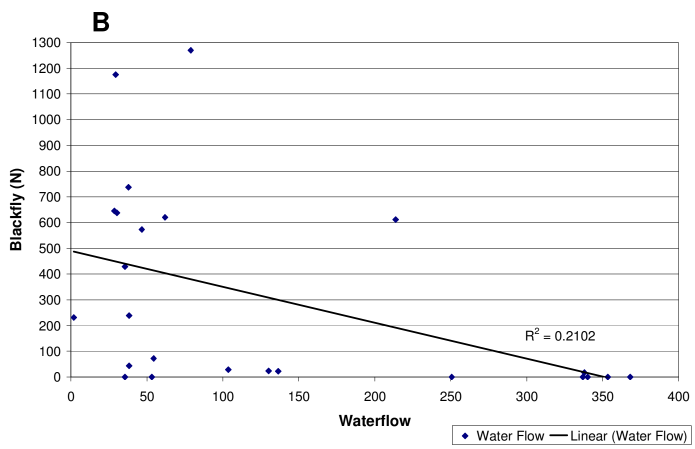


Fig. 3.4 Linear regression between immature blackfly numbers (N) and water flow (m^3/s) in the Vaal (A) and Orange (B) Rivers.

Water temperature

For the Orange River, there were high significant differences between the water temperature at the various study sites *(P = 0.001)*. For both rivers there were high significant differences in water temperature between the different seasons *(P < 0.001)*, with the minimum recorded during autumn and the maximum in summer. For the Vaal River, the water temperature ranged between 9℃ and 27℃ and for the Orange River between 8℃ and 28℃. For the Orange River, the upper part was colder than the lower part of the river. There was a significant correlation between water temperature and distance from Van der Kloof Dam (R^2 = 0.83) (Fig 3.5), i.e. from upper to lower Orange River.

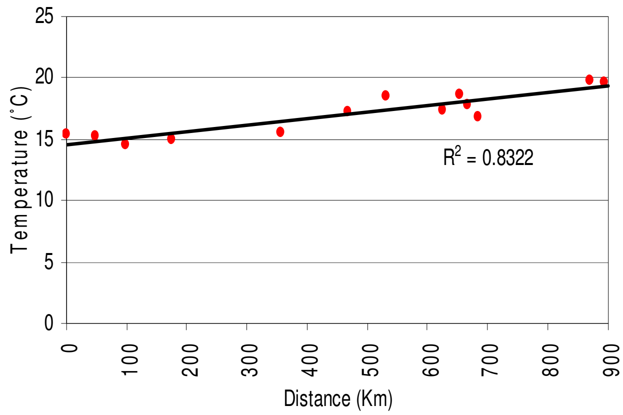


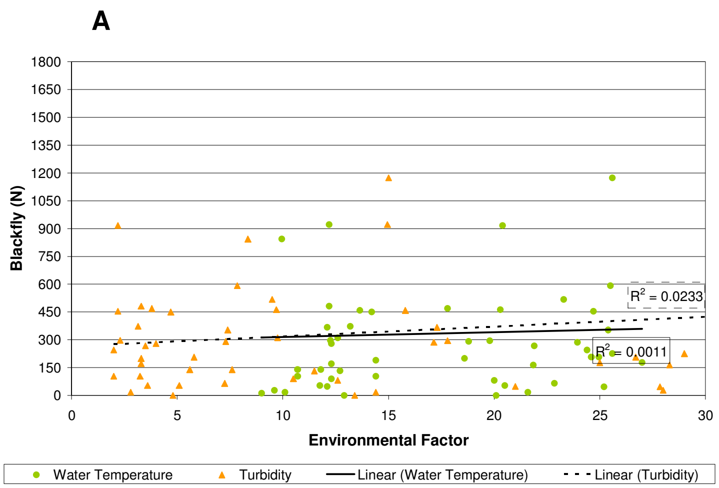
Fig. 3.5 Linear regression between water temperature and site distance from Van der Kloof Dam in the upper Orange River.

There was no significant correlation between water temperature and the numbers of immature blackflies for both the Vaal ($R^2 = 0.001$) and Orange ($R^2 = 0.01$) Rivers (Fig 3.6 A & B) although a slight negative trend was shown for the Orange River. In the summer months, with high water temperature, the blackfly numbers (Fig 3.3 A & B) were lower than in the months with colder water temperature (Fig 3.3 A & B).

Turbidity

For both the Vaal and Orange Rivers, respectively, there were significant differences of the nephelometric turbidity units (NTU) values between the study sites *($P = 0.007$, $P = 0.046$)*, as well as high significant differences for the NTU values between the seasons ($P = 0.002$, $P < 0.001$). Highest significant differences occurred in summer *($P < 0.001$)* and this was related with high flow levels.

For the Vaal, there was a slight positive correlation (Fig 3.6 A) between NTU values and blackfly numbers which was not statistically significant ($R^2 = 0.02$). In the Orange River, there was a slight negative correlation which was not statistically significant ($R^2 = 0.05$) (Fig 3.6 B).



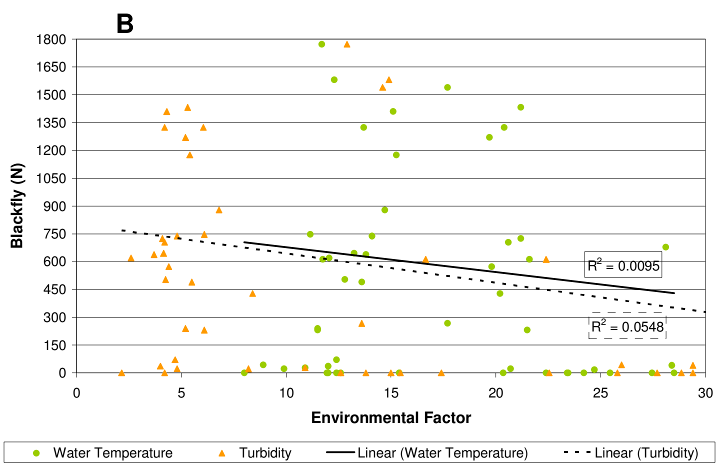


Fig. 3.6 Linear regression between water temperature, turbidity and blackfly numbers (N) in the Vaal (A) and Orange (B) Rivers.

4.2.1. Planktonic algae species

A list of all the algae species which were identified in the different seasons is given in Table 3.3. The three most abundant classes present were Bacillariophyceae, Chlorophyceae and Cyanophyceae. For the Vaal River, there were significant differences in the abundance for all these classes between the sites (Bacillariophyceae *($P = 0.009$)*, Chlorophyceae *($P = 0.046$)*,

Table 3.3: Planktonic algae species collected during the study period in the Vaal and Orange Rivers (given as no./ml).

	Vaal River				Orange River			
CLASS & species: (F = filament)	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**
CYANOPHYCEAE								
Anabaena circinalis (F)	325		255	280	390	50	220	
Aphanocapsa sp. (Colony)	200			70			70	70
Cylindrospermopsis sp. (F)	22700				750			
Merismopedia minima (col.)	560	540	1060		550	610	140	70
Merismopedia sp. (col.)	1610							
Microcystis aeruginosa (col.)	2040	540	193	220	1500	710		140
Mi. aeruginosa (loose cells)	78880	5300	840		11850	5810		
Oscillatoria sp. (F)	6525	790	680	460	610	410	248	180
BACILLARIOPHYCEAE								
Asterionella sp.			280					
Cyclotella spp. (10-20um)	4290	10930	5020	3760	2215	2160	2408	2010
Centric diatoms - small (<8 um)		420	565	850	360	260	420	420
Cocconeis sp.	1980	3250	703	330	295	420	260	250
Cymbella spp.	665	600	225	110	925	370	1248	1550
Diatoma spp.	600	600	1045	290	90		1035	1260
Gyrosigma sp.	320	680	195	110	580	250	140	80
Melosira (=Aulacoseira) granulata (F)	9320	8690	1540	460	1105	1000	590	980
Navicula sp. (pennate)	1100	1950	515		1120	390	655	1480
Nitzschia gracilis (150 um)					34200			
Nitzschia sp. (pennate)	3145	3640	1083	920	10470	560	41520	39020
Pennate diatoms (other)	1370	4610	320	80	225	560	953	870
Pinnularia sp.		70			200		215	70
Stephanodiscus hantzschii		490	1930	2140	210	1400	970	2760
Surirella ovalis			200		280			140
Synedra sp.	170		150	40	390	38050		100
CHLOROPHYCEAE								
Actinastrum hantzchii (star-col.)	250			390	300	70	70	140
Ankistrodesmus sp. (needle, 30um)	750	390	245	430		40	145	840
Carteria fornicate (4 flagellums)	3775			350	740			210
Chlamydomonas spp. (15-20um)	2395	3270	3005	1160	1485	1070	2398	1930
Chlorella sp. (3-6um)	10875	2890	3470	8070	2170	7800	10655	14780
Chlorococcum sp. (12-18um)	470	720	355	1140	790	70	70	500
Cladophora sp. (F)	800				410			
Chodatella sp.				520				
Closterium sp.	70	110	590	3220	40	1010	285	1000
Coelastrum microporum (col.)	400	40	70	70	490	140	140	180
Cosmarium sp.	700	40	70		180	150	210	140
Crucigenia tetrapedia (4x4 col.)			400				150	40
Eudorina sp. (colony)	40	70	70					
Golenkinia sp. (round with spikes)	2935	40		70		360	255	140
Mesotaenium sp.	4275	6650	1535		575	500	280	

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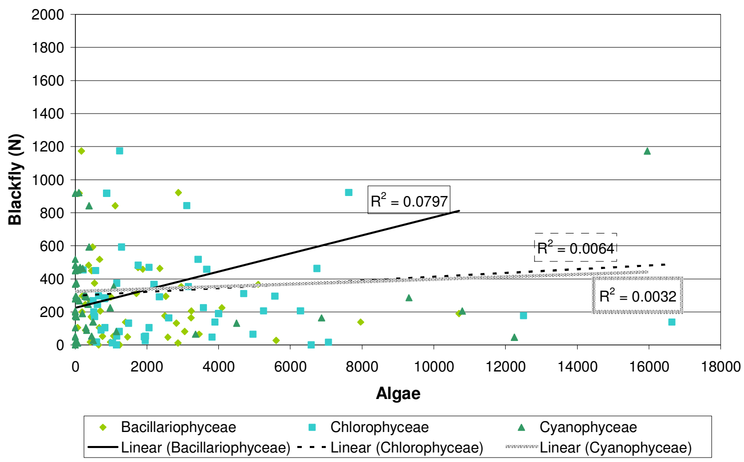
Micractinium sp. (col. round, spikes)	220	240			70		140	140
Monoraphidium arcuatum (big sickle)	1050	1020	270	630	940		2855	1700
Mo. circinale (short fat sickle)	290	320	215	470	140	70	127.5	140
Mo. contortum (S-sickle)	395	200	560	980	890	1200	2805	2390
Oocystis sp. (colony)		140	305	460	690	400	145	180
O. solitaria (loose cel, rugby ball)		200	650	150	200			
Pandorina morum (colony)	535				320			
Pediastrum sp. (colony)	615	1170	607.5	1070	980	290	395	1160
Pteromonas sp. (with sheath)	3050							
Scenedesmus spp. (colony)	3100	4820	2433	2890	1715	1040	1535	2450
Schroederia sp. (needle)					120			
Sphaerocystis sp. (colony)	600			70	70		70	
Staurastrum sp.	385	570		70			290	490
Tetrastrum sp. (4 cells with spikes)	580	370	305	2990		110	345	460
Tetraedron regulare (4-corners)	470	600	545	3010				
CRYPTOPHYCEAE								
Cryptomonas sp.	400	70	435	140	490		50	
DINOPHYCEAE								
Ceratium sp.	515	550	1525	1470				
Peridinium sp.	200		565	40	110	70	70	70
EUGLENOPHYCEAE								
Euglena sp.	455	410	143	110	70		88	320
Lepocinclis sp. (pear shape)	70	100					60	
Phacus sp.	380	470	260	40				
Strombomonas sp.		70						
Trachelomonas sp.	*880*	*610*	*1280*	*570*		280	408	400

Cyanophyceae *($P = 0.0284$)*). For the Orange River there were high significant differences for the abundance of Bacillariophyceae *($P = 0.012$)* and Chlorophyceae *($P = 0.001$)*.

There were also significant differences in the abundance of Bacillariophyceae *($P = 0.024$)* and Cyanophyceae *($P = 0.001$)* between the seasons in the Vaal River and high significant differences for Chlorophyceae *($P < 0.001$)* and Cyanophyceae *($P < 0.001$)* abundance in the Orange River.

For the Vaal and Orange Rivers there were no significant correlations between the number of immature blackflies and the abundance of these three algae classes (Fig 3.7 A & B). However, there was a slightly positive correlation between the numbers of immature blackflies and the abundance of Bacillariophyceae (*$R^2 < 0.08$*) and Chlorophyceae (*$R^2 < 0.07$*) in both the Vaal and Orange Rivers. In die Orange River there was a slightly negative correlation between the numbers of immature blackflies and Cyanophyceae (*$R^2 = 0.058$*) (Fig 3.7 B), so that in effect an increase in the Cyanophyceae abundance resulted in a decrease in the number of immature blackflies.

A



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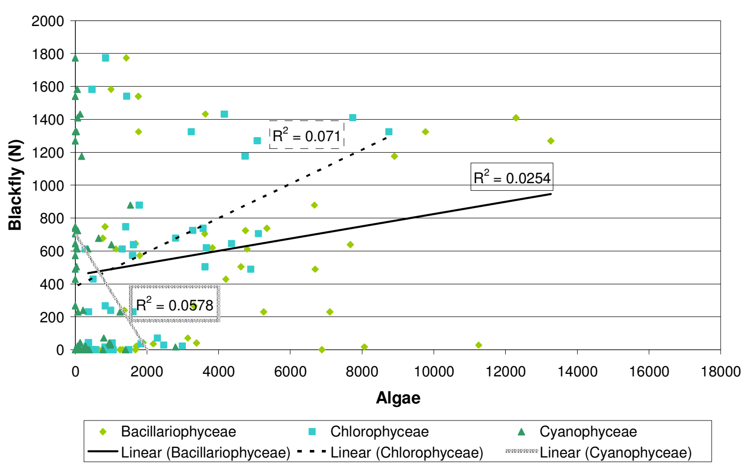

Fig. 3.7 Linear regression between algae and blackfly numbers in the Vaal (A) and Orange (B) Rivers.

1.2. Discussion

Part of the present studies were intended to determine the present state of the blackfly problem and to determine the important pest species along the Vaal and Orange Rivers. Several blackfly species were identified at two sites in each of the two rivers. *Simulium chutteri*, *S. damnosum* s.l., *S. hargreavesi, S. adersi* and *S. alcocki* were identified at Christiana and Delportshoop in the Vaal River, while *S. chutteri*, *S. damnosum* s.l., *S. adersi, S. alcocki* and *S. gariepense* were identified at Marksdrift and Ses Bridge in the Orange River. Chutter (1968) had found *S. chutteri*, *S. damnosum* s.l., *S. adersi, S. gariepense, S. nigritarsis* and *S. mcmahoni* at Warrenton in the Vaal River. In the Orange River, Palmer (1997) had found *S. chutteri*, *S. damnosum* s.l., *S. adersi, S. ruficorne, S. nigritarsis* and *S. mcmahoni* at Gifkloof. It is surprising that *S. nigritarsis* and *S. mcmahoni* were not found at the two sites in both rivers, as these species are common and are avian pests (Palmer, 1997).

Simulium gariepense is endemic to Southern Africa and was found only in the Orange River but not in the Vaal River, although Chutter (1968) had also found it in the Vaal River. In this study it could be seen that species composition can differ from site to site as not all the sites have the same formation. Comparing sites is difficult, however, as species can easily be overlooked. A further limitation is that only the pupae were identified in this study. *Simulium chutteri* was still the dominant species in the Orange River, however, as had also been indicated by Palmer (1997). From the results of this study, *S. chutteri* was not the dominant species in the Vaal River, although still present. The dominant species in the Vaal River was *S. adersi,* as had also been found by Chutter (1968).

Blackfly numbers in both the Vaal and Orange Rivers were high, which could have resulted in increasing blackfly problems. *Simulium chutteri* exploits temporary stony runs (Chutter, 1968). This biotope is found frequently in the Vaal River where diamond mining takes place and the natural river bed is changed. As there were also minimal water level fluctuations in the Vaal River, because of damming and a constant need for water, this can also increase the blackfly problem, as *S. damnosum* s.l. is favoured in these situations (Chutter, 1968). Regular fluctuations in water level were present in the Orange River. *Simulium chutteri* is more mobile and can increase to large numbers in water that fluctuates (Chutter, 1968).

In the present study, there were high numbers of immature blackflies in the winter and spring months in the Orange River. This tendency was also found by Palmer (1997). Similarly, at some sites in the Vaal River, the numbers were the highest in autumn months. A seasonal build-up from summer to winter was also observed.

There was a gradient in the water temperature. The water temperature increased from the upper Orange to the lower part. Thus overall water temperature at Van der Kloof was lower than at Onseepkans, which was also illustrated by Myburgh (2002). The blackfly numbers in the upper Orange River were also higher than in the lower part of the river. This is an indication that the build-up of blackfly numbers in the winter months is greater in the upper part of the Orange River.

The tributaries of the Vaal River, namely the Harts and Riet Rivers, did support a blackfly community. However, these were relatively small compared to the numbers in the Vaal River. There may, therefore, be a slight contribution from these tributaries in algae composition, turbidity and blackfly numbers to the Vaal River. The Vaal River is the most important tributary of the Orange River and will, therefore, also contribute to the fauna and flora of the Orange River. The dam in the Vaal River at Douglas upstream from where the Vaal and Orange Rivers meet can minimise the Vaal's contribution of blackfly numbers to the Orange River. Furthermore, this dam can regulate the water level downstream in such a manner that the downstream rapids are exposed for lengths of time which limits the numbers of blackflies and also the blackfly species composition as species that cannot tolerate water level fluctuation will be excluded.

In parts where there were great fluctuations in water levels, the blackfly numbers were lower. This is especially evident for Bloemhof in the Vaal River and Van der Kloof in the Orange River. The Port Arlington gauging station is located just below the sampling site at Bloemhof. The water levels showed that there were high variations at this site. There were also low blackfly numbers compared to the next study site downstream, at Nkolo (57 km from the dam). This trend was also seen for the Orange River at the Dooren Kuilen gauging station. There were also low blackfly numbers at the Van der Kloof site, followed by high numbers at the next site at Fluitjieskraal (48 km from the dam). This indicated that the water fluctuations at the Bloemhof and Van der Kloof dams were not sufficient to have any adequate affect further downstream.

Palmer *et al*. (2007) developed guidelines for integrated control of blackfly pests along the Orange River. Based on modelling of flow data, a flow manipulation scenario was recommended. This scenario stated that on day one the discharge from Van der Kloof Dam needed to be reduced to an average of 35 m^3/s for twelve days in July. Also on day seven the Buchuberg dam needed to be emptied and on day thirteen Buchuberg needed to be closed with further reduction in the release from Van der Kloof Dam to an average of 25 m^3/s for thirteen days. This indicated that planned reduction in water flow is needed, for water flow manipulation to be used.

The present study also indicated a slightly negative correlation between waterflow and blackfly immature abundance, although this was not significant. The higher the waterflow, the lower the blackfly immature abundance. However, it may have been that higher waterflow made sampling more difficult so that sampling could not really be done in the faster flowing parts of the rivers. Palmer (1997) suggested that blackfly immature abundance increased in faster flowing water.

Regarding the algae, Baccillariophyceae, Chlorophyceae and Cyanophyceae were found in high numbers in both the Vaal and Orange Rivers. These three classes had also been found in the Vaal River by Chutter (1968) and in the Orange River by Palmer (1997).

For Baccillariophyceae and Chlorophyceae, there were positive correlations with blackfly numbers. This is not surprising, as blackfly larvae are filter feeders. Therefore, as the food source increases, the numbers of blackflies are also likely to increase. There was a slightly negative correlation between the blackfly numbers and the Cyanophyceae numbers in the Orange River, however, so that in effect an increase in the Cyanophyceae abundance resulted in a decrease in the number of blackflies. Palmer *et al.* (2007) indicated that *Microcystis* spp. blooms caused *S. chutteri* to be replaced with *S. damnosum* s.l.. This can be promising when targeting species like *S. chutteri. Microcysti*s spp. are in the Cyanophyceae group and were also found in both the Vaal and Orange Rivers. In the Vaal River, there was no influence on blackfly numbers, however, there were also not many *S. chutteri* present at the two sites where pupae were identified.

Chutter (1968) had also indicated that benthic algae seem to lower the available attachment areas for blackflies and this can also play a role in limiting the blackfly larval populations. It appears, therefore, that algae affect blackfly numbers, as previously reported by Chutter (1968) in the Vaal River and Palmer (1997) in the Orange River.

1. BLACKFLY PARASITES AND OTHER AQUATIC INVERTEBRATES

1.2. Introduction

A wide range of predators and parasites can be regarded as the natural enemies of blackflies (Burton & McRae, 1972; Peterson & Davies, 1960). These natural enemies can have a considerable influence on the population structure of blackflies (Werner & Pont, 2003). All life stages of blackflies are attacked by these natural enemies (Werner & Pont, 2006).

Blackfly predators can range from invertebrates, birds, fishes to mammals (Werner & Pont, 2006). There are also invertebrate predators such as Acari, Aranea, Hydrozoa, Amphipoda and insects (Crosskey, 1990). The most important in this chapter are the insect predators. In the insect group there are a wide range of predators, namely: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Megaloptera, Odonata, Plecoptera and Trichoptera (Crosskey, 1990; Palmer, 1997).

Some of these insects are only predators in their immature stages (Crosskey, 1990). With the Plecoptera, Megaloptera and Trichoptera, predation is only between the immature stages of both predator and prey. In the case of Odonata and some Diptera, however, the adults are also predators and these can prey both on the adults and immature backflies (Crosskey, 1990; Peterson & Davies, 1960; Werner & Pont, 2003; Werner & Pont, 2006). Trichoptera, primarily of the families Hydropsychidae, Rhyacophilidae and Limnophilidae, are seen as the most important of blackfly predators (Burton & McRae, 1972; Kuralova & Olejnicek, 1985; Schorscher, 1993; Werner & Pont, 2006). Cannibalism among *Simulium* spp. larvae has also been reported by Burton (1971). No single predator is specially adapted to feed only on blackflies. Although blackflies do form an important food source of many predators, predation is still a random contact between predator and prey (Crosskey, 1990).

Parasites and pathogens can also play a role in the population structure and control of blackflies. These include viruses, bacteria, fungi, protozoa and nematodes (Palmer, 1997). The bacterium *Bacillus thuringienses* var*. israelensis* is a good example, as it is already used as a biological control agent (Palmer, 1997).

In this study, surveys were undertaken to identify possible predators and other biological control organisms. It was established whether there are any agents that can potentially be used for the biological control of blackflies. Emphasis was placed on predation and parasitism of the aquatic stages of blackflies. Among parasites, focus was placed on protozoa and nematodes, mainly Microspora protozoa and Mermithid nematodes.

1.2. Materials and methods

1.2.1. Blackfly parasites

The blackfly larvae collected at the various sites, using the 10-point visual ranking system described in the previous chapter, were preserved in 70 % ethanol and then inspected for parasites. Focus was placed on nematodes and protozoans.

2.2.1. Composition of predators and other aquatic invertebrates

The South African Scoring System (SASS5) (Dickens & Graham, 2002) was used for collection of aquatic invertebrates, which is suitable for the assessment of river water quality and river health. Example of the SASS5 Score sheet is given in Appendix 7. Samples were collected from two biotopes, namely stones and vegetation. For the stone biotope, stones in the current were sampled by means of a hand net. The net was placed downstream of the stones that were monitored. The stones were then disturbed for approximately two minutes. The dislodged biota was carried by the current into the net. For the vegetation biotope, the marginal vegetation in the current was sampled. A total length of approximately two meters of vegetation was sampled by pushing the net into the vegetation. All the invertebrates collected were preserved in 70 % ethanol for classification.

3.2.1. Data analyses

All parasite and predator abundance data that were collected at the sites were analysed to determine whether data records differed between seasons using the statistical software Gen Stat (2003). The parasite data were analysed using the chi-square test with Yate's continuity correction. The *P* value was two-sided; *P* value < 0.05 was considered as significant.

For predator abundance, repeated measures ANOVA was applied to all the data. Where the data passed the normality test, standard (parametric) methods were used and the Tukey test was applied. In the case where the data did not pass the normality test, nonparametric methods were used and the Friedman test was applied.

The abundance of blackfly larvae was also correlated with predator and parasite abundance using a linear regression analysis. The Vaal, Riet, Modder and Harts Rivers were analysed as one unit and the Orange River was analysed on its own.

1.2. Results

1.2.1. Parasites of blackflies

Mermithidae

In the Vaal River, blackfly larvae were infected with Mermithidae nematodes in low numbers (<1%) during all the seasons in both the stones and vegetation biotopes except in the summer (Table 4.1a) when no blackflies were infected. The infection prevalence in the Vaal River was low and there were no significant seasonal differences (*Chi-square = 0.001,* df = 1, $P > 0.60$,) between the infection prevalence in the stones biotope. In the vegetation biotope with the higher infection prevalence, there were, however, significant differences (*Chi-square = 4.49,* df = 1, $P < 0.03$) between the seasons.

In the Orange River, higher infection rates were recorded in winter and spring (between 5-10%), also in both stones and vegetation, with highest infection in autumn (81.5%) on stones (Table 4.1a). However, this accrued after treatment with *B.t.i.* implemented by the Department of Agriculture (Appendix 6). In summer only one infected larva was collected in the stones biotope and none in the vegetation. There was high infection prevalence in the Orange River (Table 4.1), with significant differences (*Chi-square = 4.02,* df = 1, $P < 0.04$,) between the seasons in both the stones and vegetation. Except for the vegetation biotope there were no significant differences ($P = 0.40$, df = 1, *Chi-square = 0.72*) between autumn and winter. The infection prevalence of this parasite is higher in the Orange River than in the Vaal River

When the blackfly larvae numbers were correlated with the numbers of blackfly larvae infected with Mermithidae, no significant correlation could be found in either river (Fig 4.1 A & B). In the Vaal River, however, there was a negative correlation between blackfly larvae numbers and blackfly larvae infected although this was not significant for both vegetation ($R^2 = 0.006$) and stones ($R^2 = 0.002$) biotopes (Fig 4.1 A).

Table: 4.1 Numbers of blackfly larvae, Mermithidae (A) and Microspora (B) (percentage of blackfly larvae infected is given in brackets)

A

	Stones		Vegetation	
	Blackfly larvae	Nematode Mermithidae	Blackfly larvae	Nematode Mermithidae
Vaal River				
Summer	583	0	2314	0
Autumn	1268	1 (0.1)	3989	3 (0.1)
Winter	689	2 (0.3)	2510	25 (0.1)
Spring	1214	2 (0.2)	2464	11 (0.4)
Orange River				
Summer	251	1 (0.4)	482	0
Autumn	81	66 (81.5)	2502	137 (5.5)
Winter	3677	207 (5.6)	5802	347 (6)
Spring	7093	471 (6.6)	5586	555 (9.9)

B

	Stones		Vegetation	
	Blackfly larvae	Protozoa Microspora	Blackfly larvae	Protozoa Microspora
Vaal River				
Summer	583	0	2314	1(0.1)
Autumn	1268	73 (5.8)	3989	19 (0.5)
Winter	689	73 (10.6)	2510	70 (2.8)
Spring	1214	48 (4)	2464	94 (3.8)
Orange River				
Summer	251	5 (2)	482	0
Autumn	81	45 (55.6)	2502	59 (2.4)
Winter	3677	2 (0.5)	5802	26 (0.5)
Spring	7093	94 (1.4)	5586	3 (0.1)

Microspora

Blackfly larvae were infected with micospora protozoans in all the seasons on both the stones and vegetation biotopes in both rivers, except during summer in the stones biotope for the Vaal River and in the vegetation biotope for the Orange River (Table 4.1B). There were significant differences (*Chi-square = 3.79*, df = 1, *P < 0.05*) in infection prevalence between the seasons for both the stones and vegetation biotope in both the rivers. Only in the stones biotope in the Orange River, were there no significant differences (*Chi-square = 0.39*, df = 1, *P = 0.53*) in the infection prevalence between summer and spring.

In the stones biotope, for both the Vaal and Orange Rivers, there was higher infection prevalence (0.1-55.6%) than in the vegetation biotope (0.1-3.8%). The infection prevalence in the Vaal River for both the stones and vegetation biotope were higher than in the Orange River, except for autumn in the stones biotope in the Orange River where the infection prevalence was 55.56% (Table 4.1B). This was the highest recorded infection, as well as the lowest recorded blackfly numbers for both the rivers. However, sampling in autumn was done after control with B.t.i. was implemented by the Department of Agriculture (Appendix 6) and this can be linked to the low blackfly larvae numbers in the Orange River.

There was no significant correlation between the blackfly larvae numbers and the larvae infected with Microspora (Fig 4.2 A & B). The only negative correlation was found in the Orange River (stones biotope) but it appears to be negligible (Fig 4.2 B).

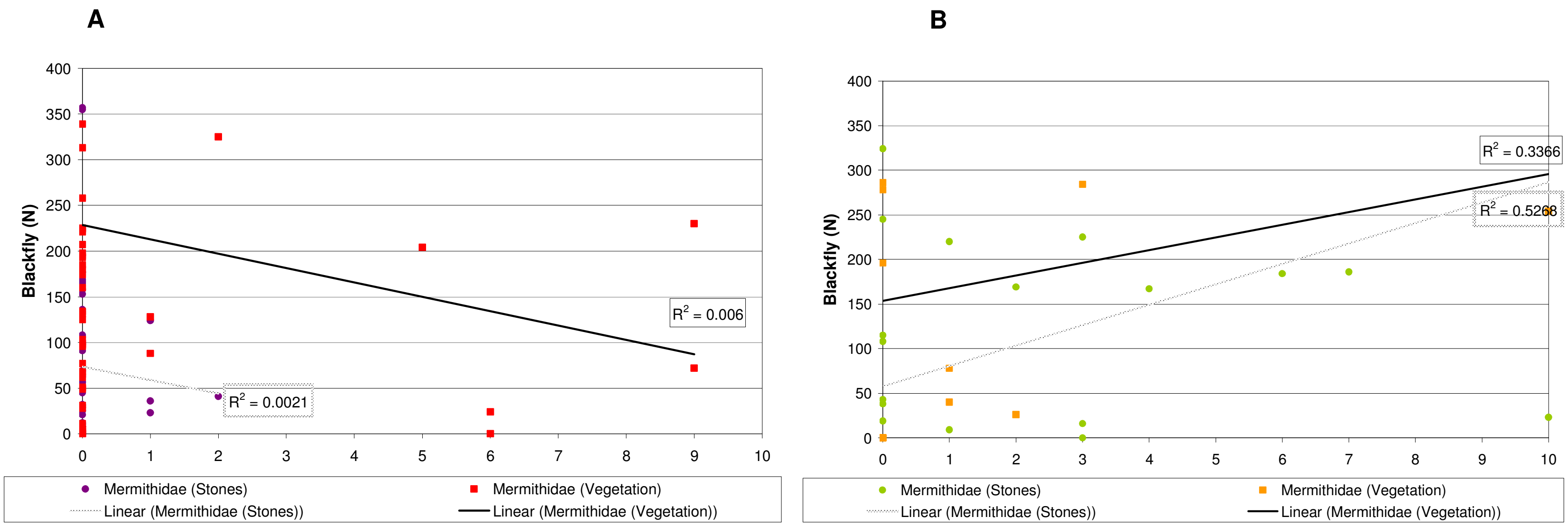

Fig. 4.1 Linear regression between Mermithidae parasite abundance and Blackfly abundance in the Vaal (A) and Orange (B) Rivers.

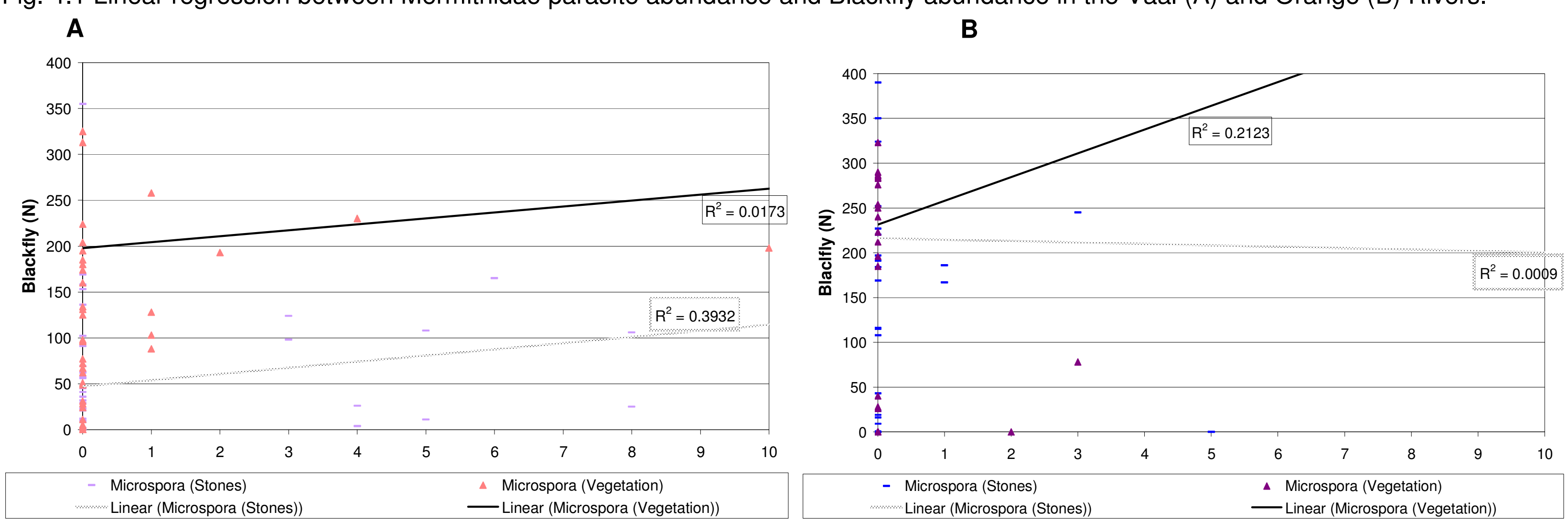

Fig. 4.2 Linear regression between Microspora parasite abundance and Blackfly abundance in the Vaal (A) and Orange (B) Rivers.

2.2.1. *Predators and other aquatic invertebrates*

A list of all the predators and other aquatic invertebrates collected is given in Table 4.2. Porifera (sponges) were also present on stones at all the sites in all the seasons but these were not collected. The predator families collected were Baetidae, Coenagrionidae, Aeshnidae, Hydropsychidae, Gyrinidae, Chironomidae and Muscidae. Baetidae, Hydropsychidae and Chironomidae were collected in the highest numbers throughout the seasons at the majority of the sites (Table 4.2). Coenagrionidae and Gyrinidae were collected in low numbers in almost all the seasons at more than 25% of the sites, within a specific season (Table 4.2). Aeshnidae, Ceratopogonidae and Muscidae were also collected in low numbers in less than 25% of the sites within a specific season and they were excluded from the analysis (Table 4.2).

Table 4.2: Number of aquatic invertebrates collected during the study period in the Vaal and Orange Rivers.

	Vaal River				**Orange River**			
Taxon	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**
ANNELIDA								
Oligochaeta (Earthworms)			4		2			
Leeches	4	15	43	16		2	27	13
CRUSTACEA								
Potamonautidae (Crabs)					1			
Atyidae (Shrimps)	57	21	34	26	61	1	1	4
HYDRACARINA								
Mites				2				
PLECOPTERA (STONEFLIES)								
Perlidae					1		11	1
EPHEMEROPTERA								
Baetidae sp. 1	244	220	370	845	167	355	146	184
Baetidae sp. 2	3	7			1	119		
Caenidae (Squaregills/Cainflies)	50	17	38	53	7		56	40
Heptageniidae (Flatheaded mayflies)	11	2	2	8	4	33	10	3
Prosopistomatidae (Water specs)							1	
Leptophlebiidae (Prongills)	20		26	1				23
ODONATA (DRAGONFLIES & DAMSELFLIES)								
Synlestidae (Chlorolestidae)(Sylphs)				1				
Coenagrionidae (Sprites and blues)	7	12	9	17	4		1	1
Aeshnidae (Hawkers & Emperors)		2					1	
Lestidae (Emerald Damselflies)	1	3						
LEPIDOPTERA								

Crambidae (Pyralidae)			1					
HEMIPTERA (BUGS)								
Belostomatidae (Giant water bugs)	3							
Corixidae (Water boatmen)	1							
Naucoridae (Creeping water bugs)	2			1	2	1		
Veliidae (Ripple bugs)	31			1	3			
TRICHOPTERA (CADDISFLIES)								
Dipseudopsidae								1
Hydropsychidae sp. 1	390	151	144	71	126	200	239	190
Hydroptilidae	13	9	94	34	6	6	21	18
Dipseudopsidae	2		5		1			
Leptoceridae							3	4
COLEOPTERA								
Elmidae/Dryopidae (Riffle beetles)		1		11	28		86	18
Gyrinidae (Whirligig beetles)	19	16	9	8	43	10	11	5
Hydraenidae (Minute moss beetles)			1		1		2	
Hydrophilidae (Water scavenger beetles)					1			
DIPTERA (FLIES)								
Ceratopogonidae (Biting midges)			1	1			1	2
Chironomidae (Midges)	80	118	419	261	47	140	667	1014
Muscidae (House flies, Stable flies)			1	31			3	3
Simuliidae (Blackflies)	754	2055	1238	1204	54	382	1852	3128
GASTROPODA (SNAILS)								
Ancylidae (Limpets)	5	3	6	11	2		5	15
Lymnaeidae (Pond snails)	7		4	1				
Physidae (Pouch snails)	7	1	1	8				
Viviparidae ST	4							
PELECYPODA (BIVALVES)								
Corbiculidae			1					
Sphaeriidae (Pills clams)	14	7	3	15	37	1	15	39

Baetidae

Baetidae nymphs were collected at almost all the sites and during all the seasons. They were the third most abundant invertebrates collected. They were found in both the stones and vegetation biotope and there were no significant differences ($P > 0.13$) in the Baetidae nymph abundance between the biotopes. In both the Vaal and Orange Rivers, in the vegetation biotope there were significant differences in Beatidae nymph abundance between the sites ($P < 0.02$) as well as the seasons ($P < 0.01$). There were also significant differences in the stones biotope in Beatidae nymph abundance between the sites ($P < 0.02$) for both the Vaal and Orange Rivers. There were no significant correlations between blackfly immature and Baetidae nymphs abundance for both stones and vegetation biotopes in both rivers (Fig 4.3 A1& B1 and Fig 4.4 A1 & B1).

Coenagrionidae

Coenagrionidae larvae were collected in low numbers in the stones and vegetation biotopes in the Orange River. In the Vaal River, low numbers were collected in the vegetation biotope, and low numbers in the stones biotope. There were thus significant differences ($P < 0.01$) in the Coenagrionidae larvae abundance between the stones and vegetation biotopes for the Vaal River. There were also significant differences ($P = 0.01$) in the Coenagrionidae larvae abundance between sites in the vegetation biotope for the Vaal River. In the Orange River, there were no significant differences ($P > 0.2$) in Coenagrionidae larvae abundance found between either the sites or seasons for both the stones and vegetation biotopes. Because of the low Coenagrionidae abundance in the Vaal and Orange Rivers, only the Coenagrionidae larvae that were collected in the vegetation biotope for the Vaal River were included in the regression analysis. There were no significant correlations between blackfly immature and Coenagrionidae larvae abundance (Fig 4.3 B2).

Hydropsychidae

Hydropsychidae larvae were collected during all the seasons at all the sites in both the Vaal and Orange Rivers. Hydropsychidae larvae were also collected in both the stones and vegetation biotopes. There was significantly ($P < 0.01$) higher abundance of Hydropsychidae larvae in the stones biotope than in the vegetation biotope in both rivers. There were also significant differences in the Hydropsychidae larvae abundance between the sites ($P < 0.01$) as well as the seasons ($P = 0.03$) in the stones biotope in the Vaal River. In the Orange River, there were only significant differences in the Hydropsychidae larvae abundance between the seasons ($P <0.01$) in the vegetation biotope. There were no significant correlations between blackfly immature and Hydropsychidae abundance (Fig 4.3 A1& B1 and Fig 4.4 A1 & B1) in both the stones and vegetation biotopes for both the rivers.

Gyrinidae

Gyrinidae adults were collected in low numbers in the vegetation biotope only in both the Vaal and Orange Rivers during all the seasons, except during spring in the Orange River. Only in the Orange River were there significant differences in the Gyrinidae adult abundance between the seasons ($P = 0.02$) in the vegetation biotope. Gyrinidae larvae were collected in low numbers in the stones as well as the vegetation biotopes for both rivers during all seasons, except in the summer in the Orange River. Because of this, there were significant differences in the Gyrinidae larvae numbers in the stones biotope between the seasons (P = 0.01) for the Orange River. In the Vaal River, there were only significant differences in the Gyrinidae larvae numbers in the stones biotope between the sites (P = 0.01). There were slight negative correlations between blackfly immature and Gyrinidae adult abundance (Fig 4.3 B2 and Fig 4.4 B2) in the vegetation biotope in both the Vaal ($R^2 = 0.002$) and Orange ($R^2 = 0.012$) Rivers, but these were not significant.

Chironomidae

Chironimidae larvae were the second most abundant invertebrates collected, after blackfly larvae. Chrinomidae larvae were collected at all sites during all seasons. The highest abundance was recorded in the Orange River in the stones biotope, so that there were significant differences in the abundance of Chironomidae larvae between the stones and vegetation biotopes ($P < 0.01$). In the Vaal River's vegetation biotope there were also significant differences in the abundance of Chironomidae larvae between the sites ($P = 0.03$) as well as the seasons ($P = 0.01$). In the Orange River's vegetation biotope there were only significant differences in the Chironomidae larvae between the sites ($P = 0.02$) while in the stones biotope there were significant differences between the seasons ($P < 0.01$). There were no significant correlations between blackfly immatures and Chironimidae larvae abundance (Fig 4.3 A1& B1 and Fig 4.4 A1 & B1) in both the stones and vegetation biotopes in both the rivers.

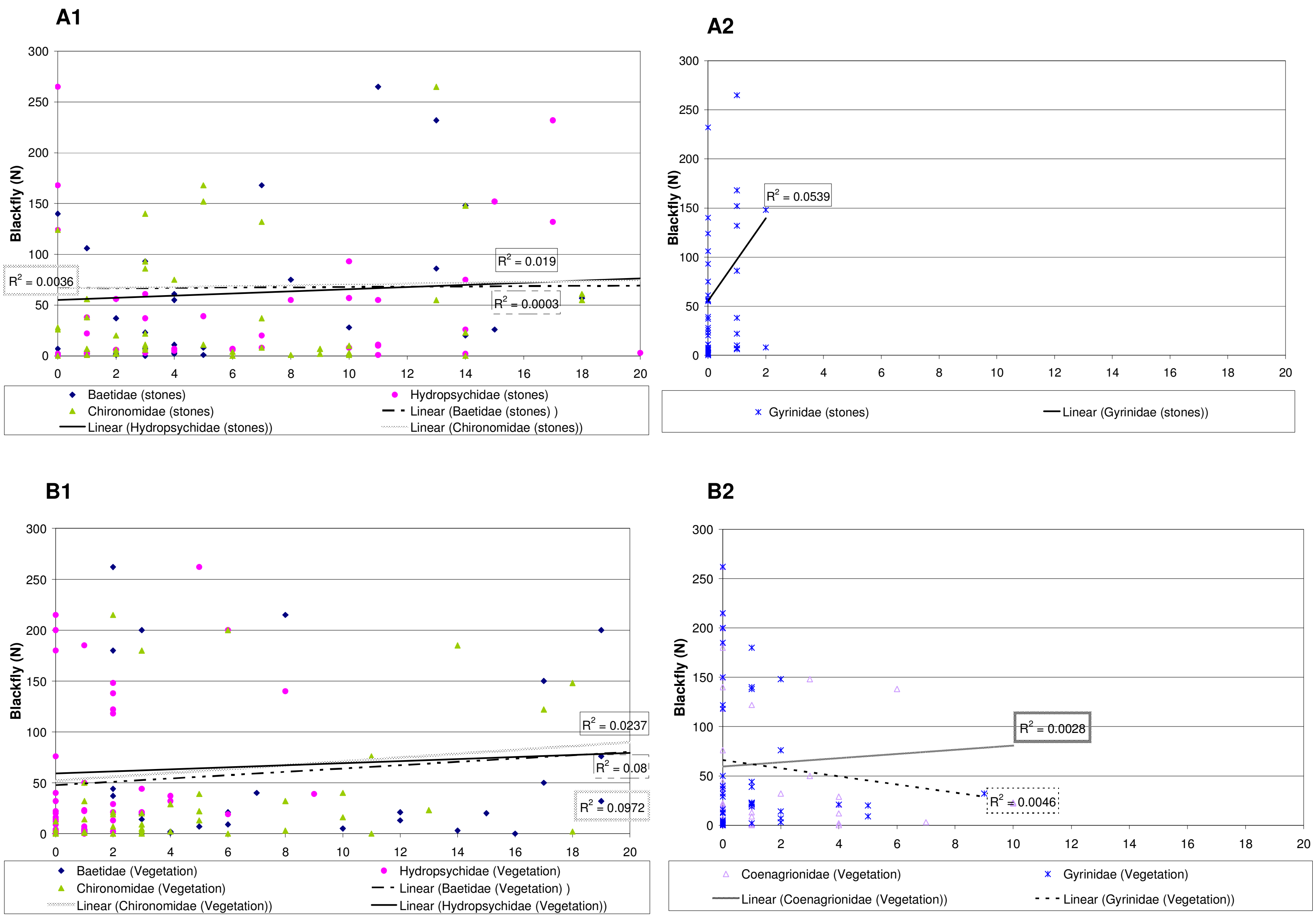

Fig. 4.3 Linear regression between predators and blackflies in the stones (A1 & A2) and vegetation (B1 & B2) biotopes for the Vaal River.

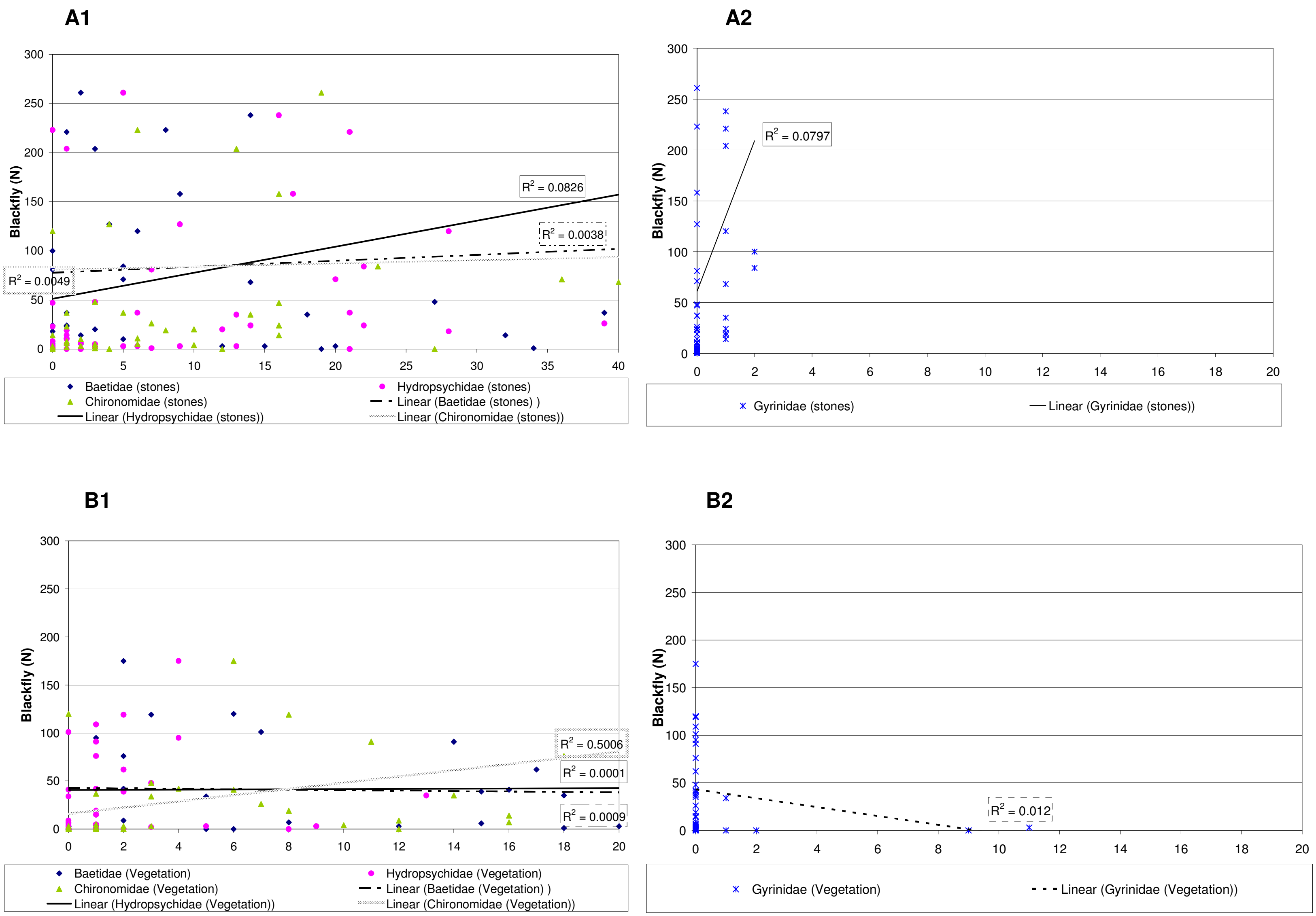

Fig. 4.4 Linear regression between predators and blackflies in the stones (A1 & A2) and vegetation (B1 & B2) biotopes for the Orange River.

1.2. Discussion

The present work identified various predators and parasites that could potentially play a role in the biological control of blackflies in both the Vaal and Orange Rivers. In both rivers, blackflies were infected with Mermithidae nematodes and Microspora protozoans. In the Vaal River the infection prevalence in natural conditions was the highest for Microspora. These protozoa are ingested by their hosts as resistant spores and develop into obligate parasites inside the cells of host organs and tissues (Crosskey, 1990; Nascimento, Figueiró, Becnel & Araújo-Coutinho, 2007). Some Microspora are seen as specific parasites to blackflies but they are not blackfly species specific (Crosskey, 1990; Palmer, 1997). Infection can cause high mortality in blackfly larvae and can prevent the larva from pupating (Palmer, 1997; Kim & Adler, 2005). Miscrosporidia can, therefore, be seen as a potential biological control agent.

Nematodes also have the potential to be a biological control agent (Webster, 1980). The highest infection prevalence of Mermithidae occurred in the Orange River. The Mermithid nematodes have a free-living adult that inhabits the same stream as the blackflies (Kim & Adler, 2005). The parasitic juvenile nematode penetrates the blackfly larva, where it will grow to the adult state and then exit the blackfly larvae through the body wall (Crosskey, 1990). Some nematodes will stay in the blackfly larvae as it passes through metamorphoses and only exit the adult blackfly (Crosskey, 1990). Infection with Mermithids does not prevent the larvae from developing into an adult and also does not prevent the adult from taking a blood meal. Most infected larvae die, however (Crosskey, 1990; Palmer 1997). According to Palmer *et al.* (2007) it appears that Mermithid nematodes are host-specific.

During the present study, Mermithidae and Microspora infections were the highest after treatments with *B.t.i.* This is an indication that these parasites can possibly be used as a control parallel to *B.t.i.* However, laboratory trials need to be done to test the influence of higher numbers of these parasites on the blackfly community.

The majority of invertebrate fauna in the Orange River are filter feeders (Palmer 1997). This was also found during this study for both the Vaal and Orange Rivers. Because of this, there is some competition between the blackflies and other filter-feeding organisms. In some cases it is not only for the food source but also for attachment sites. For example, sponges can not only filter out large quantities of food, but can also occupy large areas of attachment sites. This can influence the blackfly numbers in the area. Some Hydropsychidae (Caddisflies) construct tunnels and nets for feeding on suspended matter. The nets also reduce attachment sites and some of these also compete with blackflies for food.

The predators are, however, the organisms that influence blackfly numbers the most. Large numbers of Hydropsychidae, Baetidae and Chironomidae were collected at most of the sites and also in most seasons. This agrees with findings from Palmer (1997) in the Orange River and Chutter in the Vaal River (1968). However, none of these family groups had a significant effect on the blackfly numbers in either the Vaal or Orange Rivers. Although they have been reported to be predators, Baetidae and Chironomidae are not regarded as important (Palmer 1996). Hydropsychidae is seen as a important predator (Chutter, 1968; Burton & McRae, 1972), and has been recorded to reduce blackfly numbers (Palmer, 2007; Schorscher, 1993). This is contradictory to the results of this study where it was found that Hydropsychide had no effect on blackfly numbers. In the Great Fish River, Coetzee (1982) found that as the Trichoptera numbers increased, the blackfly density decreased. In the present study, only Gyrinidae had negative correlations with blackfly numbers, although these were not significant. However, the numbers of naturally occurring Gyrinidae populations were probably too small to have a significant impact on the massive numbers of blackflies.

Coetzee (1982) also concluded that at high blackfly abundance, they cannot possibly be controlled by predation, but at low blackfly abundance predation must have a substantial effect. A similar conclusion can be reached in this study, where the blackfly numbers are just too high for

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the predators to have any profound effects. However, it seems that both the natural populations of predators and parasites may have a limited effect on the blackfly population. The effect of an artificial increase of these predators needs to be investigated in the future.

1. GENERAL DISCUSSION

1.2. Orange River

In order to obtain an improved understanding of the current state of the blackfly problem along the Orange River, telephonic questionnaires were conducted with livestock farmers to elucidate what the public views were concerning blackfly annoyance. All the farmers indicated the existence of a blackfly problem and 52% gave it a severe rating (9-10). The majority indicated that the blackfly annoyance levels increased after the first summer rains and that the annoyance was the highest in the summer months, November and December. The majority also indicated that they were not aware of any product that can be used to protect their animals from blackfly attacks. Furthermore, the farmers implied that they were willing to participate in any control strategy that will alleviate the blackfly problem.

In-depth surveys that were done in the Orange River showed there were large numbers of breeding sites from Van der Kloof Dam to Onseepkans. Five blackfly species were identified at Marksdrift and Ses Bridge, namely *S. chutteri*, *S. damnosum* s.l., *S. adersi, S. alcocki* and *S. gariepense. Simulium chutteri* was the most abundant species, making up 95% of the species composition at these two sites. Blackfly larvae and pupae were furthermore found in high abundance at six of the thirteen sites monitored, which can be regarded as high-risk zones. These are Fluitjieskraal Bridge, Hopetown, Marksdrift, Sishen Bridge, Raap en Skraap and Onseepkans. There were also high numbers of immature blackflies in the winter and spring months, with lower water temperatures. This could be an indication of a seasonal build–up, as was also found by Palmer (1997). From the large numbers of immature blackflies collected at these sites, and the indication of the farmers, it seems that blackflies can still be considered as a serious pest along many parts of the Orange River.

Despite the continuous treatments with *B.t.i* in the Orange River, the present high numbers of blackflies is an indication that the current control strategy is not working effectively and that there are definite shortfalls. Many factors can influence the action of *B.t.i.* and any control strategy. Environmental factors such as waterflow and turbidity are important, as they influence the working of *B.t.i.* and the blackfly numbers.

During the summer, the Orange River showed high water levels and flow and also high levels of suspended solids. High flow means higher velocity of water and also high water levels mean more breeding sites. Therefore, high water level and flow will lead to subsequent increases in blackfly numbers. Higher water levels and flow also mean difficulty to monitor, as the numbers of blackflies cannot accurately be determined. Also, control with *B.t.i.* could not be done during this time as the water levels and turbidity were too high, which also led to the build-up of larvae.

Another factor that can influence the working of *B.t.i.* and is also important in the control of blackflies, are planktonic algae. Three classes of these algae were found to be the most abundant, namely Bacillariophyceae, Chlorophyceae and Cyanophyceae. Only the Cyanophyceae gave promising results, as these were the only algae that gave a negative correlation with immature blackfly abundance under natural conditions, i.e. an increase in the numbers of algae led to a decrease in blackfly abundance. Palmer (1997) indicated that blooms of *Microcystis* spp. in the class Cyanophyceae caused the replacement of *S. chutteri* populations with *S. damnosum.* This appears to be promising for the control of *S. chutteri* populations.

Parasites can also potentially have a negative effect on blackfly abundance (Chutter, 1968; Palmer *et al*., 2007). In this study it was indicated that natural infections of Mermithidae nematodes and Microspora protozoans were present in the Orange River. Mermithidae nematodes gave the highest infection prevalence in the Orange River. After application of *B.t.i.*, the infection prevalence was more than 50% for both Mermithidae nematodes and Microspora protozoans. Negative correlations were only found between Microspora protozoans and immature blackfly abundance, but they appeared to be negligible.

A wide range of organisms use blackflies as a food source (Crosskey, 1990). In this study, large numbers of Hydropsychidae, Baetidae and Chironomidae were collected. These predators had no effect on blackfly immature abundance, which is contradictory to the finding of Chutter (1968), Burton and McEae (1972) and Palmer (1996). Gyrinidae, on the other hand, were collected in low numbers but Gyrinidae was the only predator that showed a negative correlation with immature blackfly abundance so that an increase in the number of predators resulted in a decrease in blackfly numbers. Although this was not significant in nature, the high blackfly abundance must be taken into account, so that the numbers of predators were probably too small to have a significant effect on blackfly numbers.

This study showed that the Gyrinidae appeared to be the most important predator in the Orange River, which can, if artificially reared and released, potentially have a negative impact on blackfly populations.

1.2. Vaal River

During the investigation of the blackfly problem status along the Vaar River, telephonic questionnaires were conducted with livestock farmers to clarify what the public views were concerning blackfly annoyance. Seventy-eight percent of the farmers stated that there was a blackfly problem but only 24 % gave a severe rating (9-10) for blackfly annoyance. As in the Orange River, the farmers along the Vaal River also indicated that the annoyance levels increased after the first summer rains and that the annoyance was the highest in the summer months, November and December. When asked whether they used products to protect their animals, 48% indicated that they did and of these 55% indicated that these products were effective. This is contradictory to the results found in the Orange River. Furthermore, the farmers also implied that they were willing to participate in any control strategy that will improve the blackfly situation in their area.

During the survey of the Vaal River, a number of potential breeding sites were identified from Bloemhof Dam to where the Vaal meets the Orange River. Five species were identified, namely *S. chutteri*, *S. damnosum* s.l., *S. hargreavesi, S. adersi* and *S. alcocki* of which *S. adersi* was the most common species. High blackfly immature abundance was also obtained at two of the eleven sites monitored, which can be regarded as high-risk zones. These were Rietgat (Barkly West) and Schmidtsdrif. Although there was also higher blackfly immature abundance in the winter and spring months, this was not as prominent as in the Orange River. From the high blackfly abundance and the responses of the farmers, it can be concluded that there is a blackfly problem along the Vaal River, but which was not as severe as in the Orange River. There is currently no planned blackfly control in the Vaal River. Water-flow manipulation is used from time to time but there is no structured water manipulation program. Because of the lack of a structured control program, blackfly annoyance levels are high along the Vaal River.

The three most abundant planktonic algae classes in the Vaal River were Baccillariophyceae, Chlorophyceae and Cyanophyceae, none of which had a negative effect on blackfly numbers. In the Orange River, Cyanophyceae had a negative effect on blackfly immature abundance. During blooms of *Microcystis* spp., class Cyanophyceae, in the Orange River (Palmer, 1997), *S. chutteri* populations were replaced by *S. damnosum* s.l. populations. It may be that *Microcystis* spp. does not target *S. damnosum* s.l. populations the way it does *S. chutteri* populations.

The effects of natural parasitism on blackfly abundance were also investigated. Blackflies were infected with Mermithidae nematodes and Microspora protozoans, with the protozoans being the most abundant. However, only the Mermithidae nematodes had a negative effect of the natural abundance of blackfly larvae, although not significant. Because these nematodes are host-specific (Palmer, 1997) and the fact that most infected blackfly larvae die, this nematode can be considered as an important biological control agent.

As in the Orange River, there was a variety of organisms in the Vaal River that feed on blackflies. The most abundant predators were Hydropsychidae, Baetidae and Chironomidae, but none of these predators had any negative effect on blackfly immature abundance. Gyrinidae, on the other hand, was the only predator that had a negative effect on blackfly abundance. Although this negative effect was not significant, this predator was also, as in the Orange River, collected in low numbers, so that the populations were probably too small to have any significant impact on the high blackfly numbers. They may, however, have a significant impact when reared and released in high numbers.

1.2. Tributaries

The tributaries of the Vaal River were also surveyed to determine their possible contribution of blackflies, predators, algae and other non-target organisms. As the Vaal is the most important tributary of the Orange, it was investigated whether the Vaal River contributed in this regard to the Orange River. In the sample area, the Harts and Riet Rivers are the important tributaries of the Vaal River. It was concluded that these rivers did support a blackfly community and these rivers also supported similar predator, parasite and algae populations. Thus, these rivers can possibly contribute blackflies, predators and algae to the Vaal River. It was also shown that the Vaal River may possibly contribute to the blackfly, predators and algae of the Orange River. Control applied to the Orange River only will not have a sustained effect, as *S. chutteri* would re-invade this river via its tributaries.

1.2. Implications

From this study it is clear that there are a number of factors that have an impact on blackfly abundance and blackfly control. The main factors that were identified in this study were water flow, algae, parasites and predators.

Palmer *et al*. (2007) developed guidelines for the integrated control of blackfly pests along the Orange River. Through modeling of flow data, a flow-manipulation scenario was recommended, as described in Chapter 3 of this work. It was, therefore, indicated that planned reduction in water flow is needed for water-flow manipulation to be used. Such a planned system also needs to be developed for the Vaal River, should water-flow manipulation be considered as part of a control strategy. Well-planned water-level reduction at Bloemhof Dam is needed to determine the effect on current blackfly populations. At the same time the magnitude of reduction and the required time frame to achieve success should be determined, as well as for what distance in the river control will be effective.

The algae class Cyanophyceae, especially *Microcystis* spp, may also potentially play a role in biological control. However, more tests are needed before a final conclusion can be reached. Laboratory tests are needed to see whether this species can be reared in large numbers, and extensive field trials are also needed to see what effect this species has on the non-target organisms as well as the river ecosystem.

The present research implied that parasites can possibly be used within an integrated system with *B.t.i.* for blackfly control. For these parasites to be used as biological control agents, however, the feasibility of mass production and release still needs to be tested through well planned laboratory and field experiments.

Predators may also be used as potential control agents but there are many factors that can influence the efficacy of predation. Blackflies are adapted to attach themselves to areas where there is high water flow. It was also seen that, on occasion, predators were not present on the same attachment sites on a stone as blackflies, because blackflies preferred locations with faster flowing water. There is also no predator that exclusively feeds on blackflies. As Crosskey (1990) indicated, when blackflies are predated on, this is a random contact between the blackfly and the predator. As predation is a random contact and blackflies can inhabit microenvironments where they can be free of predator contact, they can have a predator-free zone where their numbers can increase exponentially.

Palmer *et al.* (2007) indicated that a biological-control program should aim to maximize the influence of natural control mechanisms and also be able to rear and release populations of natural enemies. Palmer *et al.* (2007) also indicated that none of the predator species found could be reared and released for effective blackfly control.

What is evident from this study and other similar studies done in the past on both the Vaal and Orange Rivers, is that there are limitations to both chemical and biological control approaches and that an integrated approach to blackfly control would render the best long-term results. Both the Vaal and Orange Rivers need to be controlled to have a more sustainable control strategy. An integrated biological control approach with *B.t.i* in combination with water-flow manipulation and natural enemies such as predators and parasites may be used in both rivers to give better long-term results.

It is important that a control strategy is developed with a specific river in mind. The current blackfly control programme in the Orange River, which was developed for control of *S. chutteri* along that river, is not entirely suitable for use along the Vaal River as the two rivers differ in many respects. Some of the ways in which the Vaal differs from the Orange are the following:

There are more dams and other impoundments along the Vaal River. The use for irrigation is not as extensive as in the Orange River and water is fed via canals fed from weirs, e.g. the Vaalhartz scheme. Therefore, the use of water-flow manipulation as a blackfly control option would be less disruptive to agriculture than in the Orange River. The Vaal River flows through a comparatively densely populated part of South Africa so there is a greater recreational demand on or near the river. Blackflies would, therefore, be relatively more troublesome to human activities. Also, because of the rivers' proximity to large cities, the blackflies may impact directly on associated human activities with high commercial value, e.g. conference venues, or indirectly at racing stables. Because the area along the Vaal River is less arid than the Lower Orange River, livestock production is more intensive and cattle are more important, especially dairy cattle, thus the immediate negative effect of blackfly attacks will be more apparent. Although *S. adersi* is the most common blackfly species along the Vaal River, a second species, *S. damnosum,* may also occur in pest proportions. *Simulium damnosum* does not scatter eggs on the water surface, as does *S. chutteri*, but attaches them to surfaces below the water level. This suggests that methods to control *S. chutteri* and *S. damnosum* could differ. The Lesotho Highlands Water Scheme has resulted in the regular supply of water, via the Liebenbergvlei River to the Vaal Dam, ensuring a more constant high flow of water in the Vaal River, which is favourable for the production of constantly high levels of blackflies and possibly creating new breeding sites upstream of the Vaal Dam.

It is thus important that individual control strategies are developed for the Vaal and Orange Rivers. In the Orange River, control with larvicides will be best for the preliminary control and this can then be integrated with water-flow manipulations in some parts of the river. Predators, parasites and algae may also play a role in this system. In the Vaal River, water-flow manipulation can possibly be used as the preliminary control and predators, parasites, algae and larvicides can be integrated into that system. The best control strategy for the individual rivers needs to be developed. Both rivers and their tributaries need to be controled to give a more sustainable area-wide result.

1. CONCLUSIONS

1. This study indicated that blackflies are a problem in both the Vaal and Orange Rivers. The affected parties (farmers along the Vaal and Orange Rivers) were concerned about the blackfly problem and they were willing to participate in an improved control strategy.

2. There are large numbers of breeding sites in both the Vaal and Orange Rivers, of which the high-risk zones were Barkly West and Schmidtsdrif in the Vaal River, and Fluitjieskraal Bridge, Hopetown, Marksdrift, Sishen Bridge, Raap en Skraap and Onseepkans in the Orange River.

3. *Simulium chutteri* was the most abundant blackfly species in the Orange River and *S. adersi* the most common in the Vaal River.

4. High abundance of blackfly immatures was also found in both the rivers, especially in the winter and spring months, which suggested that there was a seasonal build-up.

5. Baccillariophyceae, Chlorophyceae and Cyanophyceae were the most abundant algae classes in both the Vaal and Orange Rivers. However, only Cyanophyceae in the Orange River gave a negative correlation with blackfly immature numbers, although this was not significant.

6. Blackflies were infected with Mermithidae nematodes and Microspora protozoans in both the Vaal and Orange Rivers. Mermithidae nematodes had the highest infection prevalence in the Orange River and Microspora protozoans in the Vaal River. Mermithidae nematodes gave a negative correlation with immature blackfly abundance in the Vaal River (stones and vegetation biotopes), although this was not significant. The only negative correlation that was found in the Orange River (stones biotope), was between the Microspora protozoans and immature blackfly abundance, which appeared to be non-significant, however.

7. The predator Hydropsychidae was collected in high numbers but its presence did not have a negative effect on blackfly immature numbers. The predator Gyrinidae was collected in low numbers and there were slightly negative correlations between these predators and blackfly immature abundance, although not significant. The natural predator abundance was probably too small to have a significant impact on the massive numbers of blackflies.

8. The current control strategy along the Orange River with *B.t.i.* is not effective in the long-term so that an integrated approach with the use of *B.t.i.*, water-flow manipulation, parasites and predators may give a long-term and more sustainable solution to the control of blackflies.

9. As the two rivers differ in many ways, the control strategies for both will also require different approaches.

1. REFERENCES

Antwood, D.W., Robinson, J.V., Meisch, M.V., Olson. J.K. & Johnson, D.R. 1992. Efficacy of *Bacillus thuringiensis* var. *israelensis* against larvae of the South Buffalo gnat*, Cephia pecuarm* (Diptera: Simuliidae) and the influence of water temperature. *Journal of the American Mosquito Control Association* 8(2): 126-130.

Becker, N. & Rettich, F. 1994. Protocol for the introduction of new *Bacillus thuringiensis israelensis* products into the routine mosquito control program in Germany. *Journal of the American Mosquito Control Association* 10(4): 527-533.

Braverman, Y. 1994. Nematocera (Ceratopogonidae, Psychodidae, Simuliidae and Culicidae) and control methods. *Revue scientifique et technique, Office International des Epizooties* 13(4): 1175-1199.

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

Burgin, S.G. & Hunter, F.F. 1997. Nectar versus honeydew as sources of sugar for male and female? (Diptera: Simuliidae). *Entomological Society of America* 34(6): 605-608.

Burton, G.J. 1971. Cannibalism among *Simulium damnosum* (Simulidae) larvae. *Mosquito News* 31(4): 602-603.

Burton, G.J. & McRae, T.M. 1972. Observations on Trichopteran predators of aquatic stages of *Simulium* species in Ghana. *Journal of Medical Entomology* 9(4): 289-294.

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

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

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

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

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

Crosskey, R.W. 1986. An annotated checklist of the world of black flies (Diptera: Simuliidae). In: *Black flies, Ecology, Population Management, and Annotated World list*. Kim, K.C. & Merritt, R.W. (eds). The Pennsylvania State University, United States of America.

Crosskey, R.W. 1990. *The natural history of blackflies*. John Wiley & sons, New York.

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

Cupp, E.W., Maré, C.J., Cupp, M.S. & Ramberg, F.B. 1992. Biological transmission of vesicular stomatitis virus (New Jersey) by *Simulium vittatum* (Diptera: Simuliidae). *Entomological Society of America* 29 (2): 137-140.

Davies, D.M., Peterson, B.V. & Wood, D.M. 1962. The black flies (Diptera: Simuliidae) of Ontario. Part 1. *Adult identification and distributions of six new species. Proceedings of the Entomological Society of Ontario* 92: 70-154.

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

De Moor, F.C. 2003. Simuliidae. In: *Guides to the freshwater invertebrates of Southern Africa, Volume 9: Diptera*. Day, J.A., Harrison, A.D. & De Moor, I.J. (eds). WRC Report No. TT 201/ 02.

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

Dickens, C. & Graham, M. 2002. *South African scoring system (SASS) Version 5 Rapid bioassessment Method for Rivers.* Umgeni Water, South Africa.

Freeman, P. & de Meillon B. 1953. *Simuliidae of the Ethiopian Region*. By order of the Trustees of the British Museum, London.

GenStat®: 2003: (7th Edition) – Introduction (Editor R.W. Payne), Published 2003 by VSN International.

Gill, S.S., Cowles, E.A. & Pietrantontonia, P.V. 1992. The mode of Action of *Bacillus thuringiensis* endotoxins. *Annual Revue of Entomology* 37: 615.636.

Hansen, R.A., Hart, D.D. & Merz, R.A. 1990. Flow mediates predator-prey interactions between triclad flatworms and larval blackflies. *Oikos* 60: 187-196.

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

Howell, C.J. & Holmes, G.W. 1969. The control of Simuliidae in the Vaalharts irrigation complex. *Journal of the South African Veterinary Medical Association* 40(1): 59-67.

http://earth.google.com/

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

Kettle, D.S. 1992. Simuliidae (Blackflies). *Medical and Veterinary Entomology*, CAB International, 173-188.

Kim, S. & Adler, P.H. 2005. Multiple symbiotes in larval black flies (Diptera: Simuliidae): Associations of trichomycete fungi with ichthyosporeans, mermithid nematodes, and microsporidia. *Journal of Invertebrate Pathology* 88: 247-253.

Krueger, A. 2006. Guide to blackflies of the *Simulium damnosum* complex in eastern and Southern Africa. *Medical Veterinary Entomology* 20: 60-75.

Kuralova, M. & Olejnicek, J. 1985. Predaceous larvae of caddisflies (Trichoptera) as natural enemies of blackfly larvae (Diptera, Simuliidae) under conditions of South Bohemia. *Folia Parasitologica* 32: 375-376.

Ladle, M. & Welton, S. 1996. An historical perspective of the 'Blandford fly' (*Simulium posticatum* Meigen) problem and attempted control of the pest species using *Bacillus thuringiensis* var. *israelensis*. *Integrated Pest Management Reviews* 1 : 103-110.

Lakwo, T.L., Ndyomugyenyi, R., Onapa, A.W. & Twabaze, C. 2006. Transmission of *Onchocerca volvulus* and prospects for the elimination of its vector, the blackfly *Simulium neavei* in the Mpamba-Nkusi focus in western Uganda. *Medical and Veterinary Entomology* 20: 93-101.

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

Molloy, D.P. 1990. Progress in the biological control of black flies with *Bacillus thuringiensis israelensis*, with emphasis on temperate climates. In: *Bacterial control of mosquitoes & blackflies: Biochemistry genetics & application of Bacillus thuringiensis israelensis and Bacillus sphaericus*. De Barjac, H. & Sutherland, D. (eds). Rutgers University Press, New Brunswick, NJ.

Molloy, D.P. 1992. Impact of the black fly (Diptera: Simuliidae) control, *Bacillus thuringiensis* var. *israelensis* on chironomids (Diptera: Chironomidae) and other non-target insects: Results of ten field trials. *Journal of the American Mosquito Control Association* 8(1): 24-31.

Molloy, D.P. & Struble, R.H. 1989. Investigation of the feasibility of the microbial control of Blackflies (Diptera: Simuliidae) with *Bacillus thuringiensis* var. *israelensis* in the Adirondack Mountains of New York. *Bulletin of Society Vector Ecology* 14(2): 266-276.

Myburgh, E. 2002. *The influence of developmental temperature on the adult survival of Simulium chutteri (Diptera: Simuliidae).* MSc Thesis, University of Pretoria.

Myburgh, E., Bezuidenhout, H. & Nevill, E.M. 2001. The role of flowering plant species in the survival of blackflies (Diptera: Simuliidae) along the lower Orange River, South Africa. *Koedoe* 44(1): 63-70.

Myburgh, E. & Nevill, E.M. 2003. Review of blackfly (Diptera: Simuliidae) control in South Africa. *Onderstepoort Journal of Veterinary Research* 70: 307-317.

Nascimento, E.S., Figueiró, R., Becnel, J.J. & Araújo-Coutinho, C.J.P.C. 2007. Influence of temperature on microsporidia infections in a natural population of *Simulium pertinax* Kollar, 1832 (Diptera: Simuliidae). *Brazilian Journal of Biology* 67(3): 519-526.

Nevill, E.M. 1988. The creation of permanent blackfly problems by the construction of dams, in long-term data series relating to South Africa's renewable natural resources. In: *South African National Scientific Programmes Report No. 157,* I.A.W. MacDonald & R.J.M. Crawford (eds). CSIR, Pretoria, South Africa.

Olejnicek, J. 1986. The use of *Bacillus thuringiensis* var. *Israelensis* in the biological control of blackflies in Czechoslovakia. *Wiadomosi Parazytologigzne*, XXXII: 539-542.

Olejnicek, J. Matha., V. & Weiser, J. 1985. The efficacy of *Bacillus thuringiensis* var. *israelensis* against larvae of the blackfly *Odagmia ornata* (Meig.) (Simuliidae) at low temperatures. *Folia Parasitologica* 32: 271-277.

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

Palmer, R.W. 1993. Short-term impacts of formulations of *Bacillus thuringiensis* var. *israelensis* de Barjac and the organophoshate temephos, used in blackfly (Diptera: Simuliidae) control, on rheophilic benthic macroinvertebrates in the Middle Orange River, South Africa. *South Africa journal of Aquatic Science* 19(1/2): 14-33.

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

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

Palmer, R.W. 1996. Invertebrates in the Orange River, with emphasis on conservation and management. *South Africa Journal of Aquatic Science* 22(1/2): 3-51.

Palmer, R.W. 1997. Principles of integrated control of blackflies (Diptera:Simuliidae) in South Africa. *Water Research Commission Report No. 650/1/97.* Pretoria, South Africa.

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

Palmer, R.W. & Palmer, A.R. 1995. Impacts of repeated applications of *Bacillus thuringiensis* var. *israelensis* de Barjac and Temephos, used in blackfly (Diptera: Simuliidae) control, on macroinvertebrates in the middle Orange River, South Africa. *South African Journal of Aquatic Science* 21(1/2): 35-55.

Palmer, R.W., Rivers-Moore, N., Mullins, W., McPherson, V. & Hattingh, L. 2007. Guidelines for integrated control of pest blackflies along the Orange River. *Water Research Commission Report No. 1558/1/07*. Pretoria, South Africa.

Peterson, B.V. & Davies, D.M. 1960. Observations on some insect predators of black flies (Diptera: Simuliidae) of Algonquin Park, Ontario. *Canadian Journal of Zoology* 38: 9-18.

Priest, F. G. 1992. Biological control of mosquitoes and other biting flies by *Bacillus sphaericus* and *Bacillus thuringiensis*. *Journal of Applied Bacteriology* 72: 357-369.

Schorscher, J.A. 1993. Assessing the importance of Hydropsychidae larvae as predators of *Simulium damnosum* s.l., the vectors of river blindness in West Africa. *International Association of Theoretical and Applied Limnology* 105-115.

Van Vuuren, S.J. 1992. Hydro-power generation and the aquatic environment, a case study of the Orange River downstream of PK le Roux dam. *Electricity and Control* 11-15.

Webster, J.M. 1980. Biocontrol: The potential of entomophilic nematodes in insect management. *Journal of Nematology* 12(4): 270-277.

Werner, D. & Pont, A.C. 2003. Dipteran predators of simuliid blackflies: a worldwide review. *Medical and Veterinary Entomology* 17: 115-132.

Werner, D. & Pont, A.C. 2006. New results on diptera predators in the blackfly plague areas of Central Europe and the Caucasus. *Acta Entomologica Serbica* 131-140.

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

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1. APPENDICES

APPENDIX 1: Example of questionnaire to evaluate the blackfly annoyance on farms along the Vaal and Orange rivers.

ONDERSTEPOORT

ARC • LNR

ARC-Onderstepoort Veterinary Institute/LNR-Onderstepoort Veterinêre Instituut
Blackfly Research: Questionnaire 2006 Riviermuggienavorsing: Vraelys 2006

Please fill in and send the questionnaire to / Voltooi asseblief die vraelys en stuur terug na:

For attention/Vir Aandag **Me. C.J. de Beer**
ARC-OVI
Entomology Division (Blackfly Research Project)
Private Bag X5
0110 Onderstepoort

Name/ Naam:……………………………………………………………………………………………

Name of the farm/estate / Naam van plaas/landgoed…………………………………………...

Address/ Adres:…..………………………………………………………………………………...

……………………..………………………………………………………………………………..

Telephone and other contacts / Telefoon en ander kontakbesonderhede:…………………........

……………………………………………………………………………………………………….

1. Do you experience any problems with blackflies? / Ondervind u enige las van riviermuggies?

YES/JA	NO/NEE

2. If yes, indicate the extent of the problem in the given scale below / Indien wel, gee 'n aanduiding van die graad van die probleem op die onderstaande skaal:

1	2	3	4	5	6	7	8	9	10

3. At what time (months) of the year is the problem more evident? / Watter tyd (maande) van die jaar is die probleem die grootste?

J	F	M	A	M	J	J	A	S	O	N	D

4. Are you aware of any registered products that can be used to Protect Livestock against blackfly attack. e.g. (Delete ®, Delefe All ®, Clout ®) / Is U bewus van enige geregistreerde produkte wat gebruik kan word vir beskerming van vee teen riviermuggie aanvalle? Bv. (Delete ®, Delefe All ®, Clout ®)

……………………………………………………………………………………………………….

5. Have you used any products for protection of livestock against blackfly attacks, if YES which products have you used? / Het U voorheen enige produkte gebruik vir beskerming van vee teen riviermuggie aanvalle? Indien Ja watter produkte het u gebruik?

……………………………………………………………………………………………………

……………………………………………………………………………………………………

6. If Yes, were you satisfied or not and why not? / Indien ja was u tevrede of nie en waarom nie?

……………………………………………………………………………………………………

……………………………………………………………………………………………………

7. How far from the river is the farm/estate located? Indicate the approximate distance in kilometres.
   Hoe ver is u plaas/landgoed vanaf die die rivier Gee die geskatte afstand in kilometer.

0-20km	21-40km	41-60km	61-80km	81-100km	101-120km

8. What type of livestock is farmed at the estate? / Met watter veetipe boer u?

SHEEP/SKAPE	CATTLE/BEESTE	If other, specify/Indien ander, spesifiseer:

9. Where is the stock kept most of the time? / Waar word die vee meestal aangehou?

Feedlots / Voerkrale	Open fields / Veldweiding	If other, specify / Indien ander, spesifiseer:

10. Are you willing to become involved in the Blackfly Control Research conducted by ARC-OVI aimed at bringing control solutions for farming communities?
    Is u bereid om betrokke te raak in riviermuggienavorsing by die LNR-OVI wat daarop gemik sal wees om die probleem onder beheer te bring?

YES/JA	NO/NEE

Thank you / Dankie
Me. C.J. de Beer

For further information please contact / Vir verdere inligting kontak asseblief
Me. C.J. de Beer
Tel: 012-5299177
DeBeerC@arc.agric.za

APPENDIX 2: Satellite views and photos of the sites in the Orange River.

A

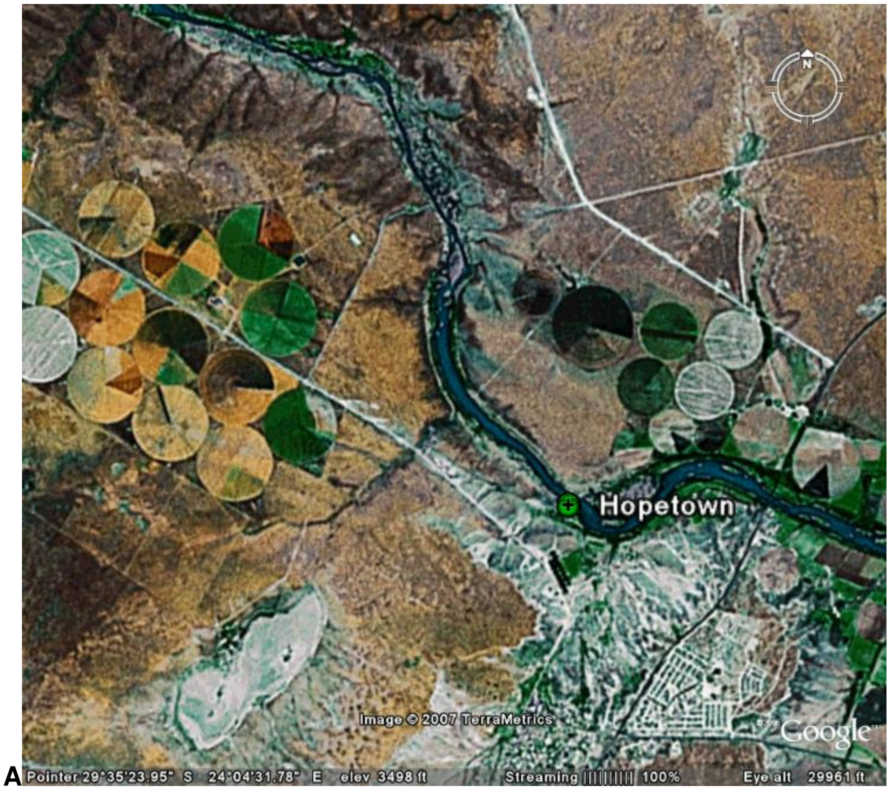
B.

Fig. 2.1 Satellite view (A) (Google Earth) and photo (B) of Van der Kloof in the Orange River.

A

B

Fig. 2.2 Satellite view (A) (Google Earth) and photo (B) of Fluitjieskraal in the Orange River.



A

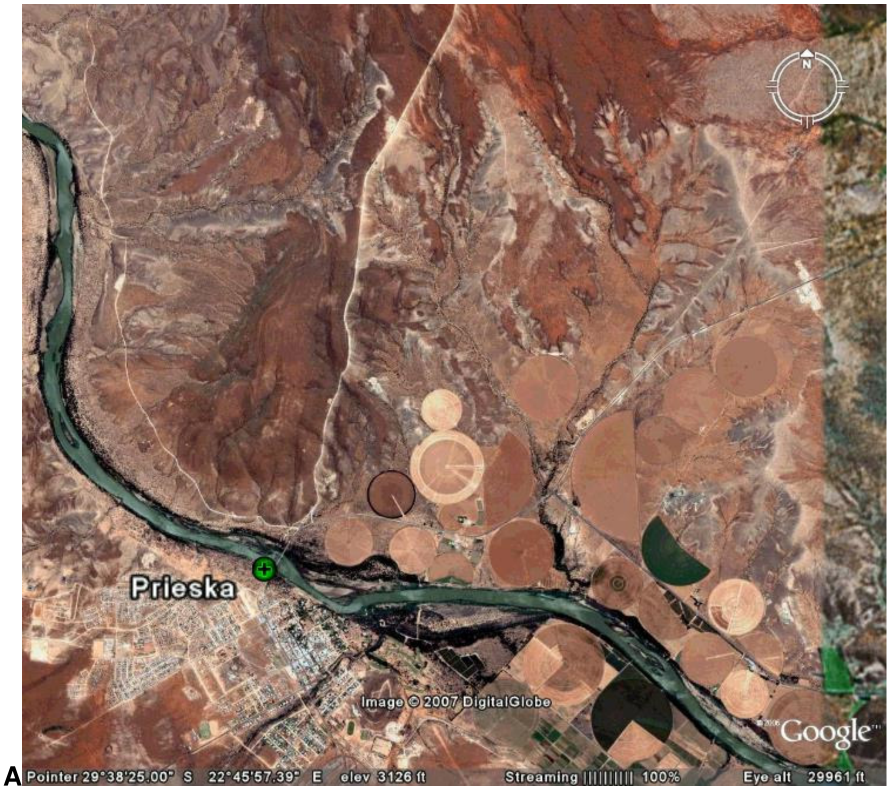
B

Fig. 2.3 Satellite view (A)(Google Earth) and photo (B) of Hopetown in the Orange River.

A


B


Fig. 2.4 Satellite view (A)(Google Earth) and photo (B) of Marksdrift in the Orange River.



A

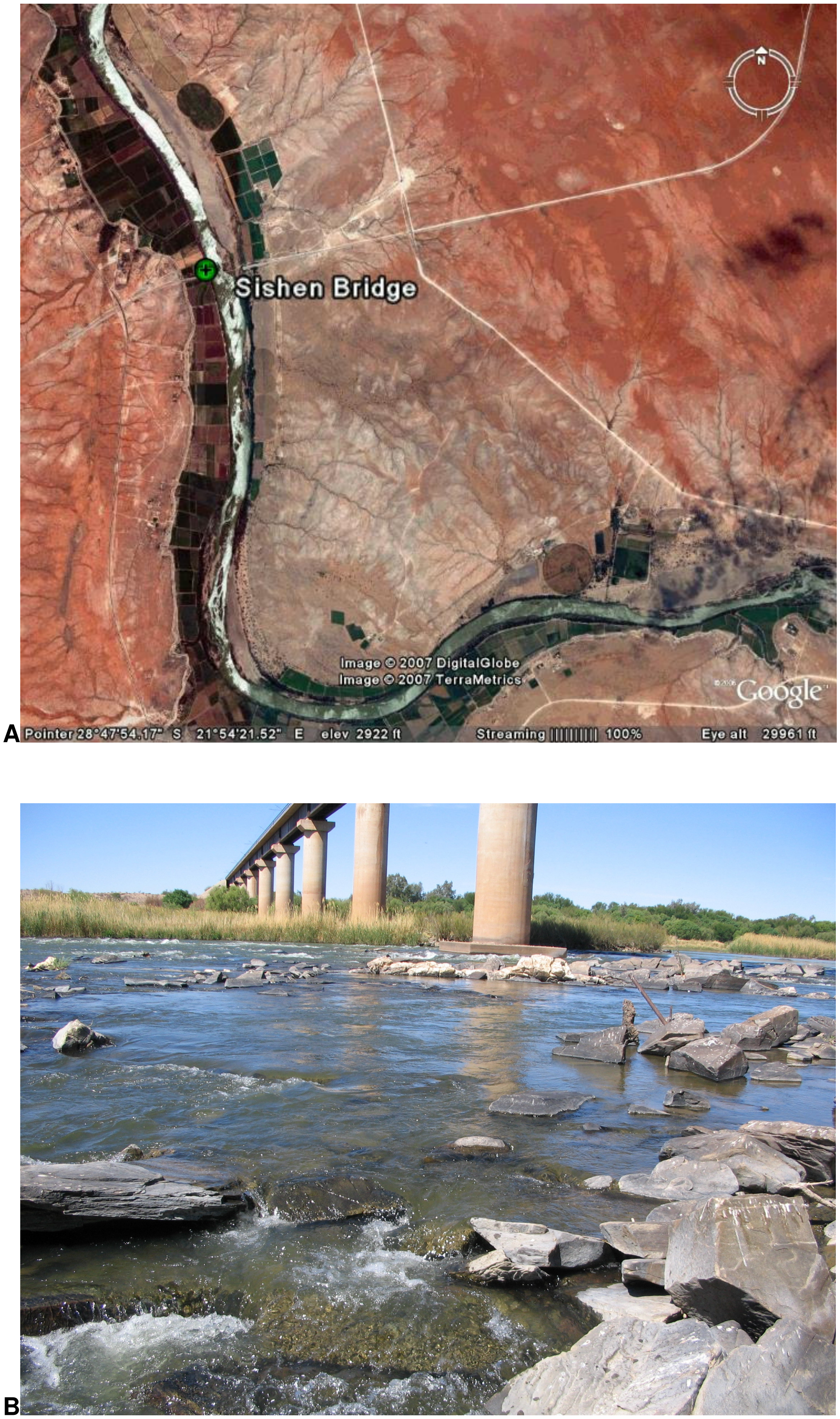
B

Fig. 2.5 Satellite view (A) (Google Earth) and photo (B) of Prieska in the Orange River.

A

B

Fig. 2.6 Satellite view (Google Earth) (A) and photo (B) of Buchuberg in the Orange River.



A

B

Fig. 2.7 Satellite view (A) (Google Earth) and photo (B) of Sishen Bridge in the Orange River.

A

B

Fig. 2.8 Satellite view (A) (Google Earth) and photo (B) of Strausbury in the Orange River.

A

B

Fig. 2.9 Satellite view (A) (Google Earth) and photo (B) of Ses Bridge in the Orange River.

A

B

Fig. 2.10 Satellite view (A) (Google Earth) and photo (B) of Kanoneiland in the Orange River.

A

B

Fig. 2.11 Satellite view (A) (Google Earth) and photo (B) of Keimoes in the Orange River

A

B

Fig. 2.12 Satellite view (A) (Google Earth) and photo (B) of Raap en Skraap in the Orange River.

A

B

Fig. 2.13 Satellite view (A) (Google Earth) and photo (B) of Onseepkans in the Orange River.

APPENDIX 3: Satellite views and photos of the sites in the Vaal River.

A

B

Fig. 3.1 Satellite view (A) (Google Earth) and photo (B) of Bloemhof in the Vaal River.


N
Nkolo Spa
Christiana
Image © 2007 TerraMetrics
Google
Pointer 27°54'00.41" S 25°10'50.62" E elev 3975 ft
Streaming |||||||||| 100%
Eye alt 29961 ft

A

B

C

Fig. 3.2 Satellite view (A) (Google Earth) and photos of Nkolo spa (B) and Christiana (C) in the Vaal River.

A

B

Fig. 3.3 Satellite view (A) (Google Earth) and photo (B) of Warrenton in the Vaal River.

A

B

Fig. 3.4 Satellite view (A) (Google Earth) and photo (B) of River Mead in the Vaal River.


Mataleng
Barkly west
Rekaofela Resort
Image © 2007 DigitalGlobe
Image © 2007 TerraMetrics
Google
Pointer 28°34'14.76" S 24°31'46.13" E elev 3717 ft
Streaming |||||||||| 100%
Eye alt 29961 ft

A

B

C

D

Fig. 3.5 Satellite view (A) (Google Earth) and photos of Rietgat (B), Mataleng (C) and Rekaofela (D) in the Vaal River.

A

B

Fig. 3.6 Satellite view (A) (Google Earth) and photo (B) of Delportshoop in the Vaal River.

A

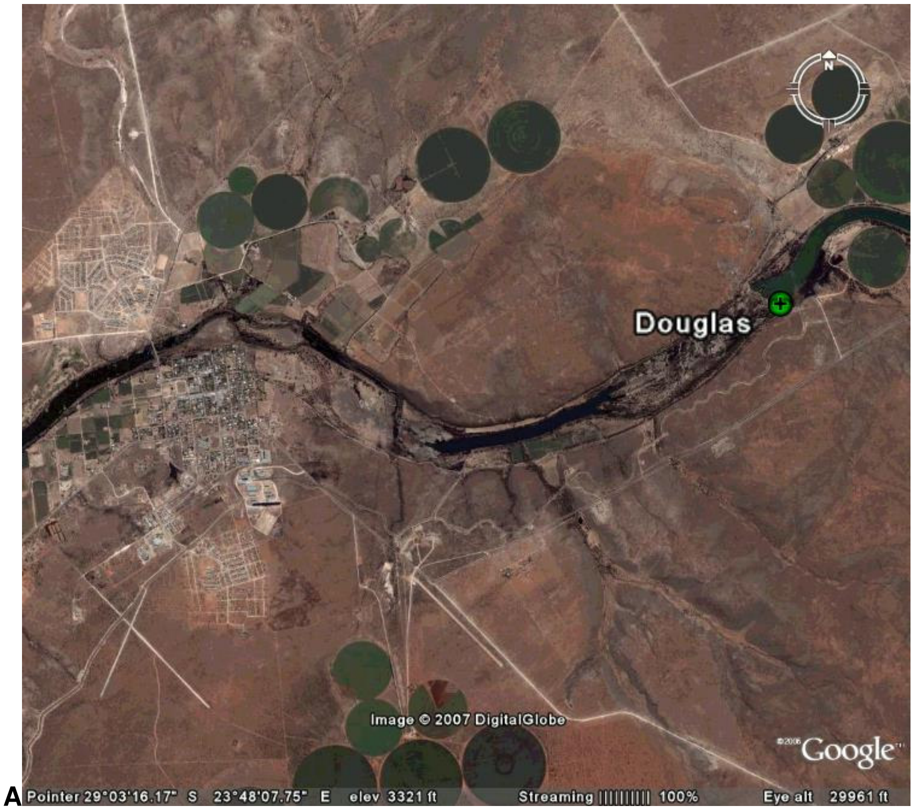
B

Fig. 3.7 Satellite view (A) (Google Earth) and photo (B) of Sydney on Vaal in the Vaal River.

A

B

Fig. 3.8 Satellite view (A) (Google Earth) and photo (B) of Schmidtsdrif in the Vaal River.



A

B

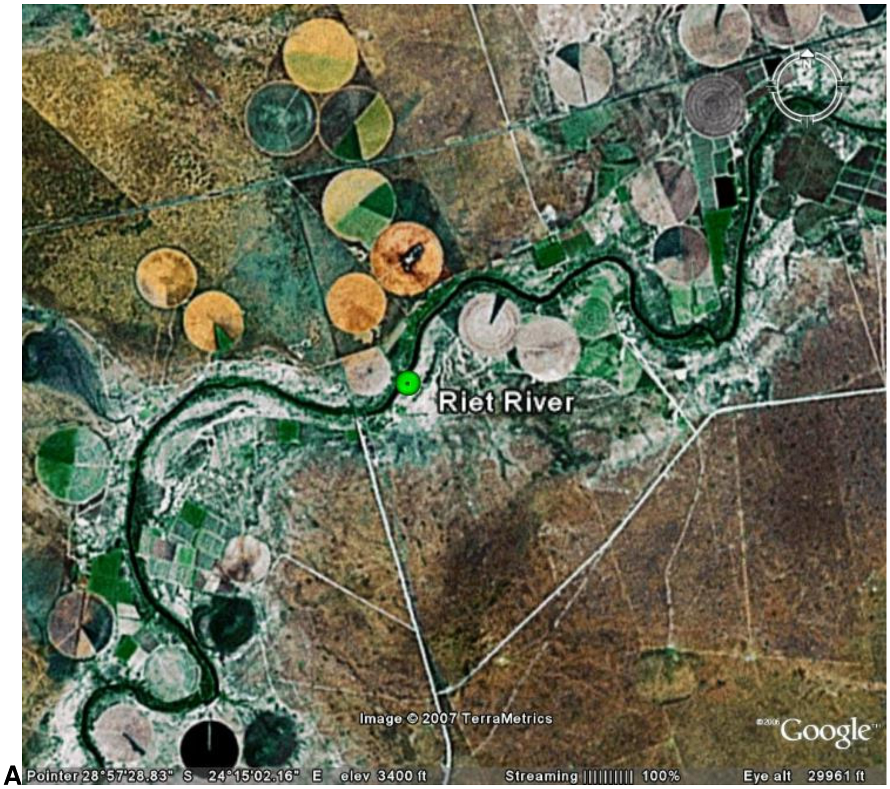
Fig. 3.9 Satellite view (A) (Google Earth) and photo (B) of Douglas in the Vaal River.

APPENDIX 4: Satellite views and photos of the sites in the Harts, Riet and Modder Rivers.

A


B

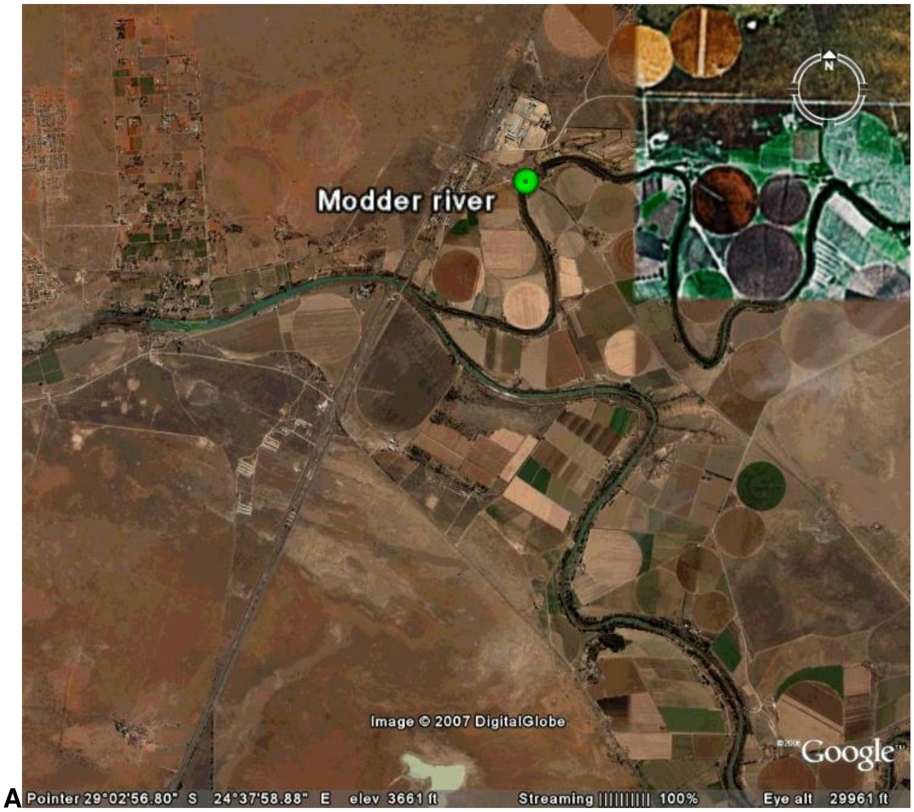
Fig. 4.1 Satellite view (A) (Google Earth) and photo (B) of the site in the Harts River.



A

B

Fig. 4.2 Satellite view (A) (Google Earth) and photo (B) of the site in the Riet River.

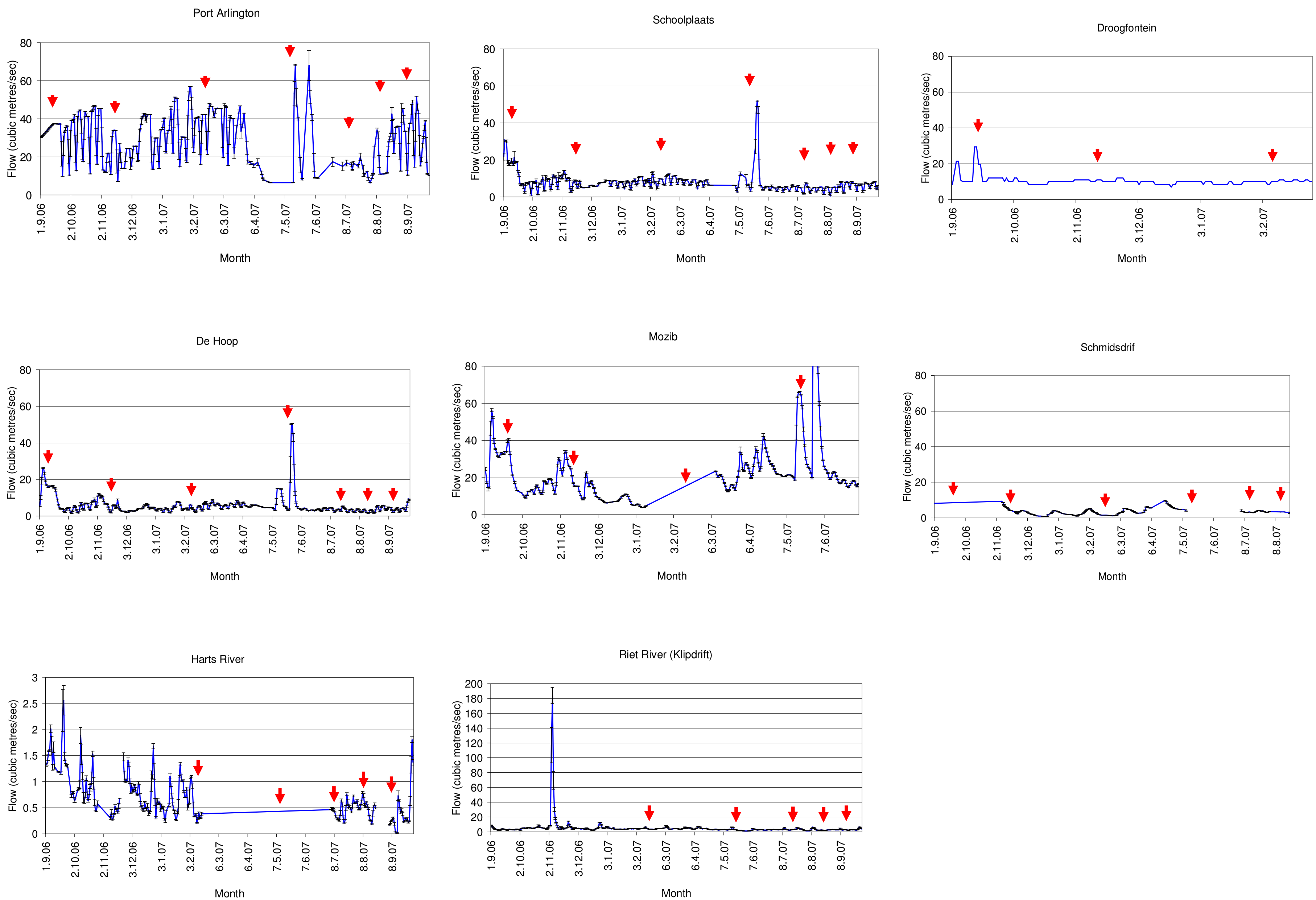


A

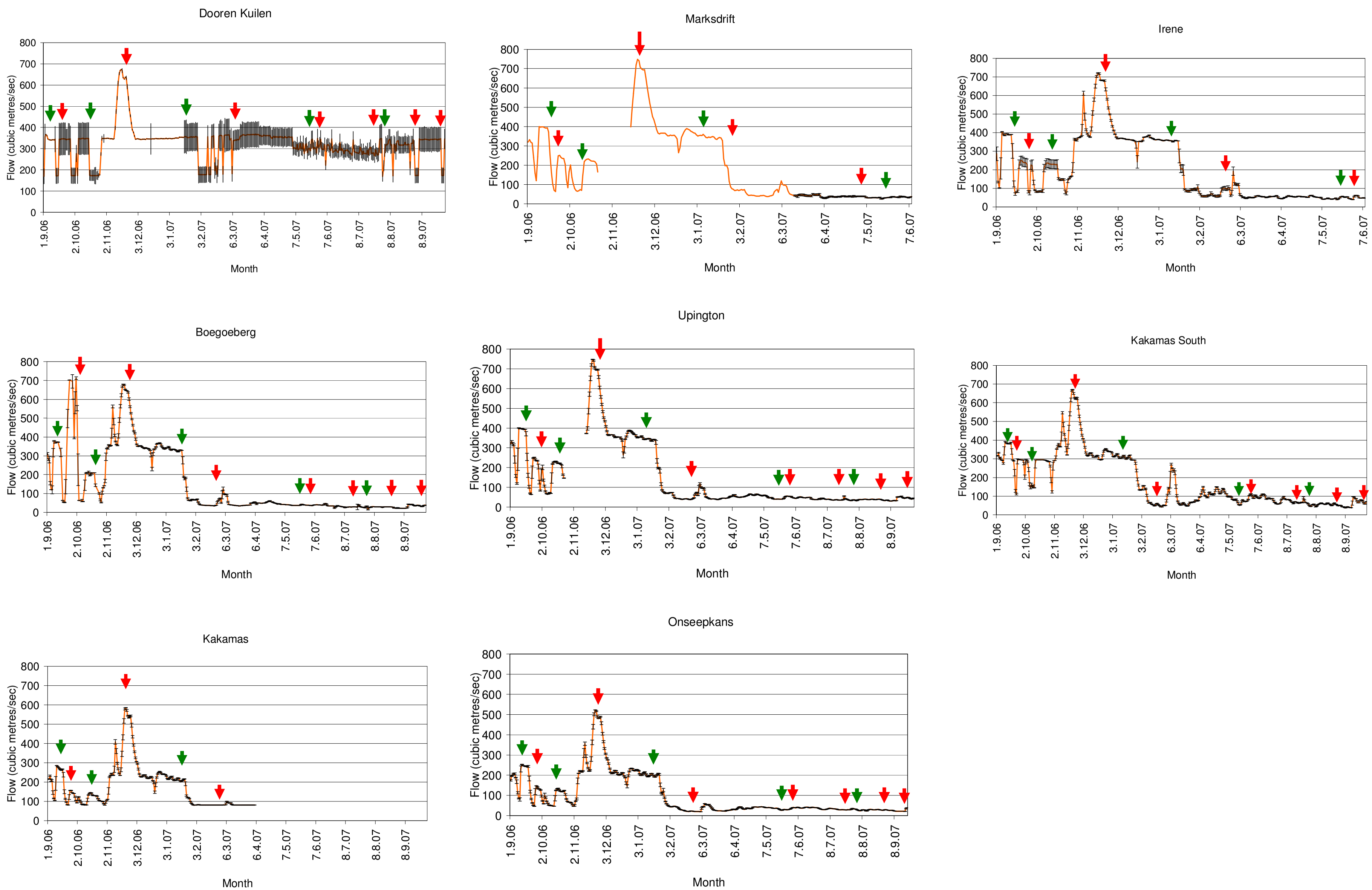
B

Fig. 4.3 Satellite view (A) (Google Earth) and photo (B) of the site in the Modder River.

APPENDIX 5: Mean daily water flow in the Vaal River at selected sites for September 2006 – September 2007. Red arrows indicate sampling occasions.



APPENDIX 6: Mean daily water flow in the Orange River at selected sites. Red arrows indicate sample visits, green arrows indicate control with B.t.i.



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APPENDIX 7: Example of the SASS Version 5 Score Sheet to be used during the Invertebrate survey of the Vaal and Orange Rivers.

SASS Version 5 Score Sheet

Date:			
Site Code:			
Ecoregion:			
Water Management Area:			
Quaternary Catchment:			
River:			
Zonation:			
Site Description:			
Collector:			
Grid (dd.mm.ss.s) **S**			
E			
Datum			
Altitude (m):			
Temp (°C):		Cond (mS/m):	
pH:		Clarity (cm):	
DO (mg/L):		Turbidity:	
Flow:		Colour:	

Biotopes sampled:	**Rating**	**Time (min)**
SIC		
SOOC		
Bedrock		
Aquatic Veg		
MVIC		
MVOC		
Gravel		
Sand		
Mud		
Hand picking/Visual observation		

Riparian Disturbance: eg, maize

Instream Disturbance: eg. sandwinning, cattle, petrol, smell etc

Taxon		**S**	**Veg**	**GSM**	**TOT**
PORIFERA (SPONGE)	5				
COELENTERATA (CNIDARIA)	1				
TURBELLARIA (FLATWORMS)	3				
ANNELIDA					
Oligochaeta (Earthworms)	1				
Leeches	3				
CRUSTACEA					
Amphipoda	13				
Potamonautidae* (Crabs)	3				
Atyidae (Shrimps)	8				
Palaemonidae (Prawns)	10				
HYDRACARINA (MITES)	8				
PLECOPTERA (STONEFLIES)					
Notonemouridae	14				
Perlidae	12				
EPHEMEROPTERA					
Baetidae 1sp	4				
Baetidae 2 sp	6				
Baetidae > 2 sp	12				
Caenidae (Squaregills/Cainfles)	6				
Ephemeridae	15				
Heptageniidae (Flatheaded mayflies)	13				
Leptophlebiidae (Prongills)	9				
Oligoneuridae (Brushlegged mayflies)	15				
Polymitarcyidae (Pale Burrowers)	10				
Prosopistomatidae (Water specs)	15				
Teloganodidae SWC	12				
Tricorythidae (Stout Crawlers)	9				
ODONATA (DRAGONFLIES & DAMSELFLIES)					
Calopterygidae ST,T	10				
Chlorocyphidae	10				
Synlestidae (Chlorolestidae)(Sylphs)	8				
Coenagrionidae (Sprites and blues)	4				
Lestidae (Emerald Damselflies)	8				
Platycnemidae (Brook Damselflies)	10				
Protoneuridae	8				
Aeshnidae (Hawkers & Emperors)	8				
Corduliidae (Cruisers)	8				
Gomphidae (Clubtails)	6				
Libellulidae (Darters)	4				
LEPIDOPTERA					
Crambidae (Pyralidae)	12				

Taxon		**S**	**Veg**	**GSM**	**TOT**
HEMIPTERA (BUGS)					
Belostomatidae* (Giant water bugs)	3				
Corixidae* (Water boatmen)	3				
Gerridae* (Pond skaters/Water striders))	5				
Hydrometridae* (Water measurers)	6				
Naucoridae* (Creeping water bugs)	7				
Nepidae* (Water scorpions)	3				
Notonectidae* (Backswimmers)	3				
Pleidae* (Pygmy backswimmers)	4				
Veliidae/M...veliidae* (Ripple bugs)	5				
MEGALOPTERA					
Corydalidae	8				
Sialidae	6				
TRICHOPTERA CADDISFLIES)					
Dipseudopsidae	10				
Ecnomidae	8				
Hydropsychidae 1 sp	4				
Hydropsychidae 2 sp	6				
Hydropsychidae > 2 sp	12				
Philopotamidae	10				
Polycentropodidae	12				
Psychomyiidae/Xiphocentronidae	8				
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae ST	11				
Glossosomatidae SWC	11				
Hydroptilidae	6				
Hydrosalpingidae SWC	15				
Lepidostomatidae	10				
Leptoceridae	6				
Petrothrincidae SWC	11				
Pisuliidae	10				
Sericostomatidae SWC	13				
COLEOPTERA					
Dytiscidae/Noteridae* (Diving beetles)	5				
Elmidae/Dryopidae* (Riffle beetles)	8				
Gyrinidae* (Whirligig beetles)	5				
Haliplidae* (Crawling water beetles)	5				
Helodidae (Marsh beetles)	12				
Hydraenidae* (Minute moss beetles)	8				
Hydrophilidae* (Water scavenger beetles)	5				
Limnichidae	10				
Psephenidae (Water Pennies)	10				

Taxon		**S**	**Veg**	**GSM**	**TOT**
DIPTERA (FLIES)					
Athericidae	10				
Blepharoceridae (Mountain midges)	15				
Ceratopogonidae (Biting midges)	5				
Chironomidae (Midges)	2				
Culicidae* (Mosquitoes)	1				
Dixidae* (Dixid midge)	10				
Empididae (Dance flies)	6				
Ephydridae (Shore flies)	3				
Muscidae (House flies, Stable flies)	1				
Psychodidae (Moth flies)	1				
Simuliidae (Blackflies)	5				
Syrphidae* (Rat tailed maggots)	1				
Tabanidae (Horse flies)	5				
Tipulidae (Crane flies)	5				
GASTROPODA (SNAILS)					
Ancylidae (Limpets)	6				
Bulininae*	3				
Hydrobiidae*	3				
Lymnaeidae* (Pond snails)	3				
Physidae* (Pouch snails)	3				
Planorbinae* (Orb snails)	3				
Thiaridae* (=Melanidae)	3				
Viviparidae* ST	5				
PELECYPODA (BIVALVES)					
Corbiculidae	5				
Sphaeriidae (Pills clams)	3				
Unionidae (Perly mussels)	6				
SASS Score					
No. of Taxa					
ASPT					

Other biota:

Comments:

Procedure: Kick SIC & bedrock for 2 mins, max. 5 mins. Kick SOOC & bedrock for 1 min. Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg $1 m^2$. Stir & sweep gravel, sand, mud for 1 min total. * = airbreathers
Hand picking & visual observation for 1 min - record in biotope where found (by circling estimated abundance on score sheet). Score for 15 mins/biotope but stop if no new taxa seen after 5 mins.
Estimate abundances: 1 = 1, A = 2-10, B = 10-100, C = 100-1000, D = >1000 S = Stone, rock & solid objects; Veg = All vegetation; GSM = Gravel, sand, mud SWC = South Western Cape, T = Tropical, ST = Sub-tropical
Rate each biotope sampled: 1=very poor (i.e. limited diversity), 5=highly suitable (i.e. wide diversity)

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