BIOLOGICAL AND CHEMICAL CONTROL OF BLACKFLIES (DIPTERA: SIMULIIDAE) IN THE ORANGE RIVER

by

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Final report to the Water Research Commission on the project **"Research on the development of an effective and environmentally safe programme for the control of *Simulium chutteri* along the Orange River".**

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EXECUTIVE SUMMARY

This project was initiated in July 1991 by the Onderstepoort Veterinary Institute (OVI) in response to complaints from farmers whose stock were being affected by the biting of pest blackflies.

The aims and objectives of this project, as originally proposed, were as follows:

(a) To determine the efficacy of commercially available, and possibly locally produced, formulations of *Bacillus thuringiensis* var. *israelensis* against blackfly larvae in the Orange River.

(b) To determine development periods of immature *S. chutteri* throughout the year (at different water temperatures) in the Orange River.

(c) To determine the prevalence of blackfly immature stages and of non-target organisms throughout the year in the upper, middle and lower Orange River.

(d) To make use of the knowledge gained, through the immatures of *S. chutteri* in the Orange River and the *B.t.i.* trials, to develop and practical and effective programme for the annual control of blackflies along the Orange River.

1. Efficacy of *B.t.i.*
This project started at the same time as the OVI's first large-scale test application of blackfly larvicides for control purposes. This research project and the OVI's Blackfly Control Project were therefore closely linked. Consequently, the aims of this project were extended to include the testing of the organophosphate, temephos. Both *B.t.i* and temephos were effective in controlling blackfly populations along the Orange River, although *B.t.i.* was suited for use in clear water, whereas temephos was suited for use in turbid water (Secchi depth < 12 cm or planktonic algae > 1500 cells/ml). The downstream "carry" of larvicides was highly variable, but increased with flow. For *B.t.i.*, "carry" was usually about 5 km at moderate flow (60-143 m^3/s), and up to 20 km at high flow (180 m^3/s). The "carry" of temephos was usually about 3 times further than *B.t.i.*.

The local production of *B.t.i.* was considered impractical. However, work conducted during this project was used for the registration of a second *B.t.i.* product (Vectobac[R] 12AS). This,

together with direct purchasing, resulted in a significant drop in *B.t.i.* prices, and the saving of approximately R 150 000 per annum (in 1993). The registration of temephos is pending further investigations on the medium-term effects of temephos on non-target organisms.

2. Development periods of *S. chutteri*

The development rates of larval *S. chutteri* were monitored following field applications of larvicides. Development largely depended on water temperature and food quality, and ranged from 7 days (in mid-summer) to 37 days (in mid-winter). Recommended time intervals between treatments ranged from 6 to 32 days. Treatments to prevent a spring outbreak should start in the last week of July. Timing of the autumn treatment should be flexible, and should be in response to any sharp increase in flow in March and April.

3. Prevalence of *S. chutteri*

A novel method of estimating the abundance of immature blackflies for use in blackfly control programmes was developed and published (Palmer, 1993). The method was based on the visual comparison of blackflies on hand-held, natural substrates, to diagrammatic representations of larvae and pupae on flat surfaces (stones) and cylindrical surfaces (trailing reeds and roots).

Two surveys of the Orange River showed that *S. chutteri* population densities were high between Marksdrift and Vioolsdrift, a distance of over 1000 km. *Simulium chutteri* was present upstream of Hendrik Verwoerd Dam, but numbers were low. Weekly sampling of *S. chutteri* larvae at Gifkloof, near Upington, showed that larval numbers were generally higher in winter than in summer. Lowest numbers occurred in autumn during algal blooms, suggesting that blackfly populations may be controlled by naturally occurring toxic algae.

4. Effects of larvicides on non-target organisms

The short-term (before-after) impacts of *B.t.i.* and temephos on non-target macroinvertebrates were studied during 8 field trials in the middle Orange River (Palmer, 1993). In all trials, blackfly larvae were the most sensitive taxa in the stones-in-current biotope to both *B.t.i.* and temephos. The number of non-target taxa (excluding other blackfly species) affected at recommended dosages was 3 for *B.t.i.* and 7 for temephos. Seven species of blackflies were found downstream of the Hendrik Verwoerd Dam, one of which (*S. gariepense*) is regarded as threatened. High-dosage applications indicated that *B.t.i.* has a wide margin of safety, whereas temephos has a narrow margin of safety. Taxa affected by temephos included the caddisfly *Cheumatopsyche thomasseti*, an important predator of blackflies.

It is concluded good control of blackflies may be obtained with minimal direct impact on the "non-target" fauna, provided recommended dosages of temephos are not exceeded. Overdosing with temephos should be strictly avoided.

5. Control of *S. chutteri*
The results of this project, and the excellent cooperation between the parties mentioned in the acknowledgements, have led to a highly successful control programme. Local farmers were kept informed about the control programme via a newsletter, called "Muggienuus", produced by the Onderstepoort Veterinary Institute. The Northern Cape Agricultural Union found that lambing percentages in 1993/94 were exceptionally good. Likewise, there were no complaints about blackflies from tourists visiting the Augrabies National between 1991 and 1994.

6. Future Research
Future research should aim to integrate chemical and biological control with other methods of control, and in doing so, provide a framework for minimising the number of treatments, and therefore the costs (both financial and environmental) of blackfly control.

7. Archiving of data
All data and invertebrates collected during this study will be housed in the Albany Museum, Grahamstown.

8. Publications and oral presentations arising from this study
Palmer, R. W. 1992. Blackfly (Diptera: Simuliidae) control in the Orange River. Abstract of Conference on Aquatic Ecosystems, Cape Town, 7-10 July, 1992. Convened under the auspices of the Southern African Society of Aquatic Scientists and the Phycological Society of Southern Africa. p 53.

Edwardes, M. and Palmer, R. W. 1993. Control of *Simulium* (blackfly) along the Orange River. Oral presentation at the *ninth congress of the Entomological Society of southern Africa*. 29 June - 1 July, Wits University, Johannesburg.

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

Journal of Aquatic Sciences. 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: x-xx.

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TERMINOLOGY

ABATE[R]	A TEMEPHOS product of SA CYANAMID.
APPLICATION	The dosage of one or more rapids for experimental purposes (see treatment).
ANAUTOGENOUS	Require a blood meal to produce eggs.
AUTOGENOUS	The ability to produce eggs without the need for a blood meal.
BENTHIC	Bottom-dwelling.
BIOLOGICAL CONTROL	The use of natural methods to control pests. For blackflies, these methods include the use of the bacterium *Bacillus thuringiensis* var. *israelensis*, predators, parasites and toxic algae.
BIOTOPE	The place where a group of organisms live. (see HABITAT.)
CARRY	The downstream distance that a larvicide application was >80 % effective.
CHEMICAL CONTROL	The use of chemically derived larvicides.
DRIFT	*Downstream movement of aquatic* insects.
DWA	Department of Water Affairs and Forestry.
HABITAT	The place where a specific organism lives. (See BIOTOPE.)
HYPORHEIC	River bed.
LENTIC	Standing water.
LOTIC	Flowing water.
MACRO-INVERTEBRATE	Large (>3 mm) invertebrate.
MERMITHIDAE	Parasitic nematodes.
MICROSPORIDIA	Parasitic protozoa.
NTO	Non-target organism. In this project, the term refers to all taxa other than *Simulium chutteri*.

OCP	Onchocerciasis Control Programme. This programme was launched in 1974 by the World Health Organisation, and aims to eradicate Onchocerciasis, or "river blindness", a disease transmitted by blackflies in west and central Africa.
OVI	Onderstepoort Veterinary Institute.
PERIPHYTON	Algae attached to stones and rocks.
TAXON (plural: TAXA)	General term for a taxonomic group whatever its rank.
TDS	Total Dissolved Solids.
TEKNAR[R]	A *B.t.i.* product from SANDOZ.
TEMEPHOS	0,0,0',0'-tetramethyl 0,0'-thiodi-p-phenylene phosphorothioate. An organophosphate. The active ingredient of ABATE[R]
SECCHI DEPTH	The depth at which a SECCHI DISC disappears when lowered into water.
SECCHI DISC	A standard disc used to evaluate the transparency of water.
SUSPENSOIDS	Fine particulate material suspended in the water column.
TREATMENT	The dosage of several rapids for control purposes. (See APPLICATION.)
TROPHIC	Pertaining to nutrition.
TSS	Total Suspended Solids: The mass of organic and inorganic material suspended in the water column and retained by a Whatman[R] GF/C filter (pore size approximately 0.6 µm). Major constituents of TSS include clay, phytoplankton and zooplankton.
VECTOBAC[R]	A *B.t.i.* product of ABBOTT LABORATORIES.
WRC	Water Research Commission.

1. INTRODUCTION

This project was initiated in July 1991 by the Onderstepoort Veterinary Institute (OVI) in response to complaints from farmers whose stock were being affected by the biting of pest blackflies.

The aims and objectives of this project, as originally proposed, were as follows:

(a) To determine the efficacy of commercially available, and possibly locally produced, formulations of *Bacillus thuringiensis* var. *israelensis* against blackfly larvae in the Orange River.

(b) To determine development periods of immature *S. chutteri* throughout the year (at different water temperatures) in the Orange River.

(c) To determine the prevalence of blackfly immature stages and of non-target organisms throughout the year in the upper, middle and lower Orange River.

(d) To make use of the knowledge gained, through the immatures of *S. chutteri* in the Orange River and the *B.t.i.* trials, to develop and practical and effective programme for the annual control of blackflies along the Orange River.

Local production of *B.t.i.* was attempted at Onderstepoort, and although the bacteria grew easily, the prevention of contamination and the formulation of a product that is effective in the field requires intensive research and major financial backing (Sutherland, 1990). It was therefore decided that commercially available *B.t.i.* products would be used in the Orange River.

Priorities of this project were to determine the relations between river conditions (Chapter 4) and the abundance of immature blackflies (Chapter 5) and their rate of development (Chapter 6). The efficacy and downstream "carry" of larvicides (Chapter 7) and the effects of larvicides on non-target organisms (Chapter 8) were examined in detail.

1.1 Blackfly control in South Africa

The first attempts to control blackflies in South Africa made use of DDT, which was used in the Vaal and Harts Rivers between 1965 and 1967 (Howell & Holmes, 1969). These trials were successful in controlling *S. chutteri*, but also killed fish and non-target invertebrates, and resulted in excessive growth of benthic algae (Howell & Holmes, 1969). DDT was not used again in the control of blackflies in South Africa, and became unpopular in other parts of the world because of the rapid development of resistance (Suzuki et al., 1963; Jamnback & West, 1970), its non-specificity (Corbet, 1958a & b; Hynes, 1960; Chance, 1970), persistence in the environment (Hynes, 1960), accumulation in food chains (Holden, 1972) and undesirability in potable water (Howell et al., 1981).

In South Africa the use of DDT to control blackflies was replaced with flow-regulation, which was considered practical, safe and cheap (Howell et al., 1981; Car, 1983a). The method involved stopping river flow for periods long enough to dry and kill blackfly larvae and pupae. This method was used successfully in the Soviet Union (Dubitskii, 1981). Flow-regulation was tested against *S. chutteri* in the Vaal River in 1977, and in the Orange River in 1978 (Howell et al., 1981). A 60 hr closure of the Vaalharts Diversion Weir reduced blackfly numbers for 30 km downstream (Howell et al., 1981). In the Orange River, a 66 hr closure at the P.K. le Roux and Boegoeberg Dams reduced blackfly numbers for 370 and 242 km respectively (Howell et al., 1981). Despite the initial success of flow-regulation in controlling blackflies, rainfall in 1987 and 1988 was higher than in previous years, and the flow in the river could not be regulated. This resulted in one of the worse blackfly plagues along the Orange River in memory. Furthermore, flow-regulation was not a practical control option for most of the Orange River because of the increased dependence of riparian agriculture on a steady water supply, the long distance downstream of impoundments (over 1000 km downstream of P.K. le Roux Dam), and the time required for drying-out of rapids. In addition, flow regulation is not target-specific, and it was feared that a drop in water level, incorrectly timed, could be detrimental to the recruitment of certain fish species by exposing fish larvae or eggs (Cambray, 1984).

Because of the problems associated with flow-regulation along the Orange River, the Onderstepoort Veterinary Institute initiated the present project on the biological control of blackflies, using the bacterium *B.t.i.*. This bacterium has been successfully used to control blackflies in numerous control programmes worldwide (Gaugler & Finney, 1982; Guillet et al., 1982; 1985; Molloy & Struble, 1989). However, *B.t.i.* is relatively bulky and would be logistically impractical to apply at flows exceeding 200 m^3/s. Furthermore, the efficacy of *B.t.i.* drops in water which is turbid or contains a high algal content (Kurtak pers.

comm.). The aims and objectives of this project were therefore extended to include the testing of the organophosphate temephos. However, the Steering Committee agreed that the aims and objectives of this research project should remain as originally proposed.

Other potential methods of controlling blackflies include the sterile-male technique and the use of parasitic nematodes, viruses, protozoa, fungi or predators (Molloy, Gaugler & Jamnback, 1980; Molloy, 1981; Poinar, 1981; Gaugler & Molloy, 1981; Gaugler, Kaplan, Alvarado, Montoya & Ortega, 1983; Lacey & Undeen, 1986; 1987). However, the evidence indicates that biocontrol agents, with the exception of *B.t.i.*, have had limited success in controlling pest blackflies.

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2. LITERATURE REVIEW

2.1 *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*)

B.t.i. is a rod-shaped, spore-forming, aerobic, gram positive bacterium found in soils (Couch & Ross, 1980). It was first isolated from dead mosquitoes found in a desert pool in Israel in 1977 (Goldberg & Margalit, 1977; Margalit, 1990). The following year it was reported to kill blackfly larvae (Undeen & Nagel, 1978), and is now used worldwide in the control of blackflies and mosquitoes. The toxic component is a protein crystal (delta-endotoxin) found in the parasporal inclusions which are produced during sporulation (Molloy et al., 1984; de Barjac, 1990; Gill et al., 1992). The crystals become active in the presence of proteases and the alkaline pH of the larval midgut (Lacey & Undeen, 1986). The toxic polypeptides interfere with osmotic balance by binding to midgut epithelial cells, and kill larvae within minutes after ingestion (Lacey & Undeen, 1986). The active ingredient (endotoxin) in a typical *B.t.i.* formulation constitutes less than 1 % of the volume. The remaining volume includes ingredients used to grow the bacteria, such as fishmeal, flour, soyabeans, casein, yeast, starch, molasses and dextrose (Dulmage, 1989). In addition, various other chemicals, such as benzene, toluene and xylene may be used to improve shelf-life, efficacy, dispersion and carry (Fortin et al., 1986). In South Africa *B.t.i.* is available for blackfly control under the trade names TEKNAR® HP-D and VECTOBAC® 12AS.

B.t.i. is a non-contact larvicide, and therefore needs to be ingested before effective. Mortality is therefore a function of the amount of larvicide ingested, which depends on particle size, the concentration of larvicide, duration of exposure and the rate of feeding. Although *B.t.i.* particles 30-50 µm in diameter in size were found to be the most effective against *S. damnosum* in West Africa, the particles settled rapidly, and smaller particles provided better downstream "carry" (Guillet et al., 1985a).

Numerous studies have examined the relationship between larvicide concentration, duration of exposure, and effectiveness of different *B.t.i.* formulations (Gaugler & Finney, 1982; Lacey & Undeen, 1984; Guillet et al., 1985c). Formulae used to calculate the volume of insecticide to be applied, based on river section, lethal dose of product used, number of particles per unit weight, ingestion as a function of temperature, species, particle size and particle flux, have been developed (Elsen, 1987). A more practical method of determining a suitable dosage is to apply a sequence of increasing severe treatments at intervals of 2-3 days until either the practical application limit is reached, or blackfly larvae are eliminated (Undeen & Lacey, 1982). According to the labels of the two *B.t.i.* products tested in this study (Teknar[R] HP-D and Vectobac[R] 12AS), toxicities were rated at 1200 International Toxic Units (ITU), equivalent to 3000 *Aedes aegypti* units. Toxicity ratings are based on bioassays with *A. aegypti*, and this may result in disparity between ITU ratings and their efficacy against blackflies. For small streams, a rough approximation of the amount of larvicide to apply may be obtained from the stream width (Undeen et al., 1984).

One of the main advantages of *B.t.i.* is its specificity against target insects, and harmlessness to vertebrates, including humans (Heimpel, 1967; Lacey, 1985a). The development of larvicidal resistance to *B.t.i.* has been considered unlikely, but recent findings indicate that resistance is occurring (Holmes, 1993; Tabashnik, 1994). The mechanism of resistance is reduced binding of the *B.t.* toxin to epithelial cells (Tabashnik, 1994). However, in West Africa, extensive use of *B.t.i.* for seven years has not produced resistance exceeding 1.5 fold among *S. damnosum* s.l..

Table 1 Advantages and disadvantages of *B.t.i.* and temephos. Data are from Heimpel (1967); Lacey & Heitzman (1985) and Sutherland (1990).

Consideration	B.t.i.	Temephos
Target specificity	excellent	poor to moderate
Public attitude	excellent	poor to moderate
Resistance	slow	likely with repeated use
Efficacy in clear water	good	poor
Efficacy in turbid water	poor	good
Carry	moderate	excellent
Agitation of spray tank required	yes	no
Shelf life	variable	excellent
Volume	bulky *	highly concentrated

* Current formulations of <u>B.t.i.</u> require roughly 2.4 times the volume of temephos.

A further advantage of *B.t.i.* is that it is effective both in cold and warm water (Morin et al., 1989), and is effective even at temperatures of 0-7oC (Colbo & O'Brien, 1984). Tests in the Vaal (Car & de Moor, 1984) and Orange Rivers (de Moor & Car, 1986) showed that *B.t.i.* was effective against *S. chutteri* larvae, although its efficacy against other species was reduced in polluted water (Car, 1984). Studies elsewhere have shown that the potency of *B.t.i.* varies greatly depending on the concentration of suspended solids (Ramoska et al., 1982; Van Essen & Hembree, 1982; Morin et al., 1989). More importantly, current formulations of *B.t.i.* are relatively bulky, requiring roughly 2.4 times the volume of organophosphate insecticides.

This means that the application of *B.t.i.* to large rivers (> 200 m^3/s) is logistically impractical (Lacey & Heitzman, 1985). Furthermore, *B.t.i.* does not "carry" as far as conventional larvicides, and therefore requires more application points. This therefore increases the cost of application. Although *B.t.i.* requires agitation during application, too much agitation may trap air and turn larvicide into a thick consistency which is difficult to apply. Another problem with *B.t.i.* concerns its shelf-life. Shelf-life depends on formulation, and great improvements have been made since early *B.t.i.* formulations, which were notoriously unstable. New formulations of *B.t.i.* can withstand exposure to tropical conditions for at least 16 months (Guillet et al., 1982b). However, because *B.t.i.* is a biological product, it is still advised that the product be used as fresh as possible. It was for these reasons that the use of the organophosphate, temephos, was also considered for use for blackfly control in the Orange River.

2.2 Temephos

Temephos is a sulfur-containing organophosphate widely used in the control of mosquitoes (Lores et al., 1985; Lee & Scott, 1989) and blackflies (Kurtak, Grunewald & Baldry, 1987). Like all organophosphate compounds, temephos affects the nervous system by inhibiting the release of the enzyme acetylcholinesterase (Chambers & Levi, 1992). This leads to the persistence and accumulation of the neurotransmitter acetylcholine (ACH) at synaptic junctions (Chambers, 1992). Although temephos accumulates in fatty tissues (Miles et al., 1976; Matthiessen & Johnson, 1978; Lèvêque et al., 1988), the accumulation does not increase indefinitely as it does with organochlorines, such as DDT (Matthiessen & Johnson, 1978). The active ingredient in a typical temephos formulation constitutes 20 % of the volume. The remaining volume is made up of emulsifiers, solvents and water. (Details of these ingredients were not disclosed by the company concerned.)

Temephos was first used for blackfly control in West Africa in 1975, and was the only larvicide used by the Onchocerciasis Control Programme (OCP) between 1975 and 1980 (Lèvêque et al., 1988). Temephos was chosen by the OCP because of its effectiveness against blackflies, low mammalian toxicity (Gaines, 1969; Laws et al., 1967) and safety for most non-target organisms, including fish (Von Windeguth & Patterson, 1966; Swabey et al., 1967; Gaines, 1969; Jamnback, 1973; Davies et al., 1978; Mohsen & Mulla, 1981; 1982). Numerous studies conclude that temephos is one of the safest organophosphates available for blackfly control (Gaines et al., 1967; Laws et al., 1967; Gaines, 1969; Kurtak, Grunewald & Baldry, 1987; Kurtak, Jamnback, Meyer, Ocran & Renaud, 1987; Wallace & Hynes, 1981). However, temephos has not yet been registered for blackfly control in South Africa.

One disadvantages of temephos is the rapid development of larvicidal resistance (Post & Kurtak, 1987). In December 1979, resistance to temephos was discovered in larvae of the *S. damnosum* s.l. complex in West Africa (Guillet, Escaffre, Ouedraogo & Quillevere, 1980; Kurtak, 1986). The use of temephos in those areas in which larvicidal resistance had developed in West Africa (21 % of the total area under control; Lacey & Undeen, 1986) was replaced by the organophosphate chlorphoxim (WHO, 1985). Two years later, resistance to chlorphoxim was discovered among species already resistant to temephos (Kurtak et al., 1982). By 1990 the OCP was using five different larvicides to control blackflies (Le Berre et al., 1990; Kurtak, 1990; Davies, 1994). The choice of larvicide varied from season to season, place to place, and depended on river profile, discharge and previous treatments (Le Berre et al., 1990). The development of larvicidal resistance may be reduced by leaving large parts of rivers untreated (Curtis, 1987) and by treating rivers intermittently (Curtis 1987; Adiamah et al., 1986). For example, after 8 years of intermittent treatment with temephos on the lower Volta River, larvae of *S. damnosum* s.l. complex showed no changes in susceptibility, whereas larvae developed resistance within 14 months of weekly treatment in the Ivory coast (Adiamah et al., 1986).

The main disadvantage of using temephos in the Orange River is that it affects a wide variety of invertebrates, including blackfly predators (see Chapter 7). The Orange River is a long river with few tributaries, and recolonisation potential is very low.

A further drawback with temephos is that studies elsewhere have shown that it does not work at low temperatures (Back et al., 1979). However, temephos shelf-life is excellent, and it retains effectiveness for over 3 years under tropical conditions (Kurtak et al., 1987).

2. 3 Blackfly species in the Orange River

When this project began it was assumed that *S. chutteri* was the only pest blackfly species in the region. It was soon discovered that *S. chutteri* was one of at least seven species of blackfly found in the Orange River downstream of the H. F. Verwoerd Dam (Table 2). Although all seven feed on livestock or poultry, not all are regarded as pests. A selected review of what is known about each of these species is given below.

Simulium chutteri Lewis

One of at least 4 members of the *S. bovis* complex (Lewis, 1964). Although this complex is widespread in central and southern Africa, *S. chutteri* is restricted to large, turbid rivers in southern Africa (Fig. 1). *Simulium chutteri* was first described

Table 2. Monthly changes in the abundance of blackfly species found at Gifkloof, near Upington, between August 1991 and March 1994. [1=present; 2=common; 3=abundant.]

	1991					1992											
	V											V					
	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
S. chutteri	1	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3
S. mcmahoni	-	-	-	-	-	-	-	-	1	2	2	2	-	1	2	2	2
S. damnosum s.l.	-	-	-	-	-	-	-	-	-	*	-	-	-	-	*	*	1
S. adersi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. gariepense	-	-	-	1	-	1	1	-	-	1	1	-	-	-	-	-	-
S. ruficorne	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. impukane	(Found once at Boegoeberg Dam before this study began.)																

	1993												1994					
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
S. chutteri	2	2	2	1	2	2	2	3	3	2	1	1	1	1	2	1	1	1
S. mcmahoni	1	*	2	*	1	1	2	2	2	1	2	1	1	1	1	1	1	1
S. damnosum s.l.	-	-	1	1	2	2	1	2	2	3	2	2	2	1	2	2	2	1
S. adersi	-	*	1	2	2	2	2	2	1	1	2	1	1	1	1	1	1	-
S. gariepense	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. ruficorne	-	-	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-

Stars (*) indicate taxa collected in the Upington area at times other than regular monthly sampling. The "V" indicates the timing of larvicide applications which affected Gifkloof.

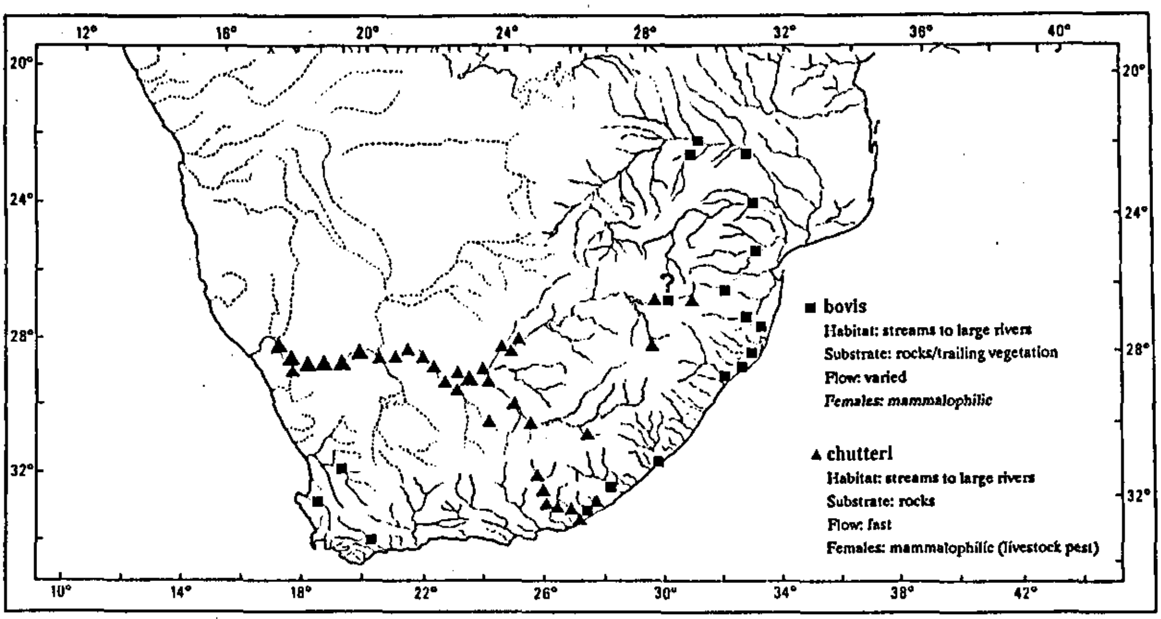

Figure 1. Map of southern Africa showing the distribution of *Simulium chutteri* and the closely related *S. bovis*.

from specimens collected by Dr Mark Chutter from the Vaal River (Lewis, 1964). It is a serious pest of livestock along the Orange (Howell et al., 1981; Car, 1983a; de Moor & Car, 1986), Vaal (Chutter 1967; 1968a; Howell et al., 1981; Car & de Moor, 1984), Great Fish (Coetzee, 1982; O'Keeffe, 1985; O'Keeffe & de Moor, 1988) and Sundays (Chutter, 1968b) rivers. It is the most common blackfly species in the middle and lower Orange River. Although *S. chutteri* was present in the Great Fish River before the opening of the Great Fish River tunnel in 1977, its numbers were low, and it was not considered a pest (Chutter, 1972). The introduction of Orange River water converted the once seasonal Great Fish River into a perennial river (O'Keeffe & de Moor, 1988), and *S. chutteri* replaced the previously dominant *S. nigritarse* s.s (Coetzee, 1982; O'Keeffe 1985; O'Keeffe & de Moor, 1988; Palmer & O'Keeffe, 1990). Similar changes in river conditions caused by the Lesotho Highlands Scheme are likely to favour *S. chutteri* in the Liebenberg tributary of the Vaal River (Chutter, 1993).

Larvae attach onto rocks and trailing vegetation where the current speed is fast (>1.0 ms^{-1}). High numbers are usually associated with fast-flowing, turbid water. During extended periods of clear conditions, they are replaced by other blackfly species (Car & de Moor, 1984). Under normal conditions, larvae have 7 instars (de Moor, 1982a), but there is evidence that they have 6 instars after floods (O'Keeffe, unpublished data). Variable numbers of instars are known for *S. vittatum* in Canada (Colbo, 1989). A guide to separating *S. chutteri* instars was prepared by de Moor (1982b). Mean mass of mature (final instar) larvae from the Vaal River ranged from 0.31 mg (in summer) to 0.89 mg (in winter)(de Moor, 1982b).

Under favourable conditions, larval densities exceed 500 000/m^2. In the Orange River, the drift at Marksdrift comprised 98.7 % *S. chutteri* larvae (Car, 1983a). The drift of *S. chutteri* larvae peaks in the early morning and late afternoon, and is higher at night than during the day (de Moor, 1982b; de Moor et al., 1986). Pupae hatch after 3 days at 23^{0}C, or 10 days at 13^{0}C (Fig. 2b; de Moor, 1982b: 94). Females are significantly larger than males (de Moor, 1982b).

Female *S. chutteri* are primarily zoophilic, although they sometimes feed on birds, including ostriches. They feed voraciously on donkeys, horses, sheep, goats, cattle, springbuck and occasionally people. Although *S. chutteri* are not known to transmit any diseases, circumstantial evidence suggests that they do. They are suspected of transmitting the protozoan parasite *Chlamydia* sp. which causes blindness in sheep, and Rift Valley Fever Virus when the disease broke out along the Orange River in 1975. Their bite is painful and may cause severe local swelling which may remain itchy for days or weeks, and lead to secondary infections. Animals stop feeding when the flies are present, and consequently loose condition, and sometimes die. Lambing percentages are significantly affected, and in 1990 the Northern Cape Agricultural Union estimated that *S. chutteri* accounted for up to R 33 million per annum in lost animal production along a 800 km stretch of the Orange River. The flies make working

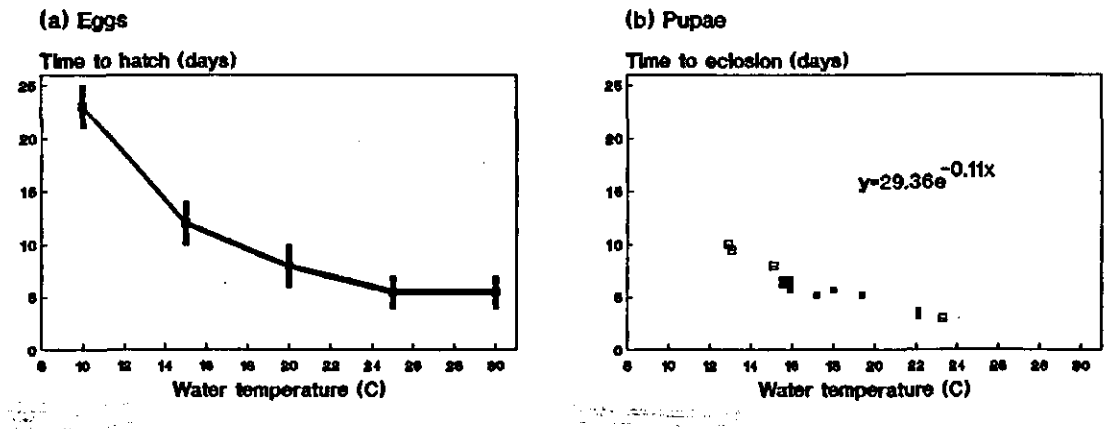

Figure 2. Development rates of *Simulium chutteri* eggs (a) and pupae (b) as a function of water temperature. Data for eggs are from Begemann (1986: 13), and for pupae data are from de Moor (1982b: 94).

outside intolerable, particularly in the early mornings and evenings. They are a nuisance at the Augrabies Falls National Park, where tourists complain about them crawling into their hair, ears, noses, eyes and mouths. Females are particularly troublesome in spring (September to November; Jordaan & Van Ark, 1990), and sometimes in autumn (March to May). Males feed on nectar, including that of the Buffalo Thorn, *Ziziphus mucronata* (Begemann, 1986). Males form mating swarms over fast-flowing water at the lips of rapids or weirs (Begemann, 1980a; de Moor, 1982b). Newly emerged females enter such swarms and copulation is completed on the water surface (Begemann, 1980a). Follicular development starts before emergence (de Moor, 1982b; Begemann, 1986), and is inhibited by mermithid nematode infections (Chutter, 1967). Females require long stretches of slow-flowing water upstream of rapids to drop eggs (de Moor et al., 1986). Eggs settle in sediments, and up to 2×10^{6} *S. chutteri* eggs per m^{3} have been found in sediment from the Vaal River (Begemann, 1980b). Eggs are 196x157 µm in size (de Moor, 1982b), and take 4 to 7 days to hatch at 25 ± 1^{0}C and 30 ± 1^{0}C, and 21 to 25 days at 10 ± 1^{0}C (Fig. 2a; Begemann, 1986). Newly emerged females from the Orange River contained 488±41SD (n=110) ovarioles, with no change in number through the year. Realised fecundity has been estimated at between 40 and 292 eggs per gonotrophic cycle (Begemann, 1980a). The number of gonotrohic cycles is not known, but in West Africa *S. damnosum* has been estimated to have up to 5 cycles (WHO, 1985: 75).

Reasons for the success of *S. chutteri*

Simulium chutteri is one of the most successful blackfly species in southern Africa. Comparisons with other blackfly species in the region show that *S. chutteri* has a unique set of characters which may account for its success (Table 3).

One possible reason for the success of *S. chutteri* is that, unlike other *Simulium* species in the area, eggs are laid on the water surface (de Moor, 1982b). Eggs settle in the sediments, where they are unaffected by fluctuations in flow (Howell et al., 1981). The rapid appearance of *S. chutteri* larvae following increases in river discharge suggest that at least some eggs enter diapause, and hatch in response to increases in flow (de Moor, 1982b; de Moor et al., 1986). A pest blackfly in Australia (*Austrosimulium pestilens*) has a similar biology, in which eggs remain viable for at least 2.5 years (Colbo & Moorhouse, 1974; 1979).

Table 3. Likely reasons for the success of *S. chutteri*.

Attribute	Consequence	Reference
Eggs		
* Eggs settle in sediments	Tolerate changes in flow	Howell et al., 1981
* Eggs probably enter diapause	Rapid response to changes in flow	de Moor, 1982b 1986
Larvae		
* Unique head-fan structure	Tolerate fast-flowing water	Palmer, 1991
* Tolerates fast-flowing water	Reduces predation and competition	de Moor et al., 1986
* Highly mobile	Tolerate changes in flow	Chutter, 1968a
* Highly mobile	Reduces predation	Chutter, 1968a
* Instar flow preferences differ	Reduces competition between instars	de Moor et al., 1986
* Growth sensitive to temperature	Rapid response to changes in temperatures	de Moor, 1994
Adults		
* Large winter females autogenous	Generations overlap in spring	de Moor, 1982b
* Mating occurs at rapids	Females emerging are mated immediately	Begemann, 1980a
* Open topography	Good visibility for host location	Crosskey, 1990

Another possible reason for the success of *S. chutteri* concerns the larval head (feeding) fans. Although the head fans of small *S. chutteri* are similar to many blackfly species, large *S. chutteri* larvae have an unusual structure in which each fan ray bears two rows of highly differentiated microtrichia (Fig. 3a). These long microtrichia (14 µm) are flexible and have curved tips which hook around the base of microtrichia on adjacent rays to form a coupling network. The network strengthens the fans, and enables *S. chutteri* to inhabit and feed in very fast-flowing water. Most other blackfly species in southern Africa, including *S. nigritarse* s.s. and *S. bovis*, have fans which bear a single row of short microtrichia (Fig. 3b). Barber

(1985) showed that *S. chutteri* are more efficient feeders in turbulent water than *S. nigritarse* s.s..

a b

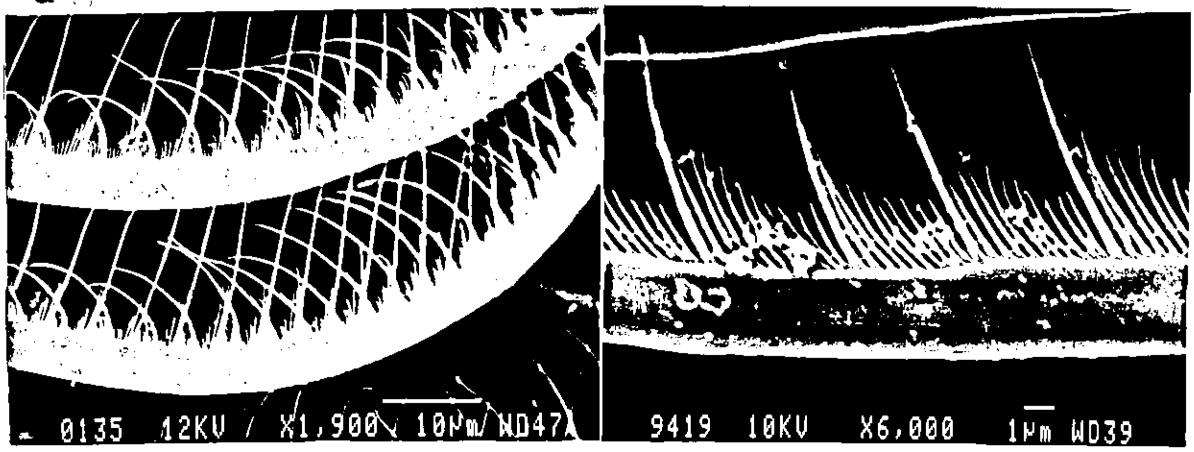

Figure 3. Feeding fan rays of final instar *S. chutteri* larva (a) and that of the closely related *S. bovis* (b). Photographs are from Palmer (1991).

The ability of *S. chutteri* to inhabit very fast-flowing water reduces competition from other blackfly species, and also reduces the chances of predation. In the Vaal River, highest densities of *S. chutteri* larvae were found where the river was narrow, and flow fluctuations greatest (Chutter, 1968a). The larvae were more mobile than other sympatric species (e.g., *S. adersi*), and it was suggested that their greater mobility accounted for their high densities in fluctuating environments. Larvae are quick to colonise newly inundated areas, an ability attributed to their high mobility and likely presence of diapause eggs. An interesting observation is that small *S. chutteri* larvae (<4th instar) settle out of the drift in areas upstream of rapids (de Moor et al., 1986). Larger larvae are usually found in very fast-flowing water (1-1.5 m/s). This difference in the microhabitat preferences between instars reduces intra-specific competition for attachment sites (de Moor, et al., 1986).

Another reason for the success of *S. chutteri* is that the growth of larvae and pupae is more sensitive to temperature compared to the closely related *S. bovis* (de Moor, 1993). Growth rates are depressed during winter, and fast in summer. They therefore respond rapidly as temperatures rise in early spring.

Water temperature has long been known to affect the size and development rate of blackfly larvae (e.g., Colbo & Porter, 1981; Rempel & Carter, 1986; 1987a & b), and *S. chutteri* is no exception (de Moor, 1982b). Winter larvae develop slowly, and are more than twice the size of summer larvae (de Moor, 1982b). Winter larvae also contain larger fat reserves compared to summer larvae (de Moor, 1982b). Females emerging in early spring are therefore likely to be more fecund than females emerging at other times of the year. Furthermore, these large females may be autogenous (Begemann, 1980a), and would therefore not be noticed by farmers (de Moor, 1982b). However, they are likely to play an important role in spring population outbreaks, and their population size is likely to dictate the size of the spring population (de Moor, 1982b). As temperatures increase in spring, large (winter) generations of *S. chutteri* co-exist with small (spring) generations, and this concentration of generations leads to population outbreaks in spring (de Moor, 1982b). The outbreaks have been aggravated by impoundments which have increased winter flows, and thus increased the numbers of large, fecund females (de Moor, 1989).

The success of *S. chutteri* may also be attributed to the fact that males form mating swarms at rapids, and females are inseminated immediately after emerging (Begemann, 1986). Furthermore, stock farming ensures that the flies have a ready supply of blood, which was presumably not the case prior to settled agriculture, when blackflies must have relied on migrating herds of game for blood. The success of *S. chutteri* may also be attributed to the open topography in which the adults are found. Blackfly pests of livestock worldwide are found in open country where good visibility is important in host location (Crosskey, 1990: 546).

A survey of the Great Fish River (Palmer & O'Keeffe, 1990) showed that *S. chutteri* were largely absent in a 25-86 km stretch of river downstream of Elandsdrift Dam, and were replaced by other blackfly taxa (*S. nigritarse* s.s., *S. medusaeforme*, *S. adersi* and *S. damnosum* s.l.). Likewise, in the Vaal River, *S. chutteri* were scarce in a 8 km stretch downstream of the Vaalhartz Weir (Chutter, 1968a; de Moor, 1982b, de Moor et al., 1986). One reason for the change in blackfly dominance downstream of impoundments may lie in the different methods of blackfly oviposition: *S. chutteri* scatter their eggs onto the water surface, whereas *S. nigritarse* s.s. and other common blackfly taxa in southern Africa, secure their eggs onto substrates (de Moor, 1982b, de Moor et al., 1986). This could mean that Elandsdrift Dam acts as a barrier to downstream colonisation of *S. chutteri* eggs, and explain their relative scarcity downstream of the dam (de Moor, pers. comm.). However, there are numerous blackfly taxa which are common in impoundment and lake outlets elsewhere, which also scatter their eggs onto the water surface (e.g., *Cnephia dacotensis*, *Simulium decorum*, *S. tuberosum*, *S. venustum*, *S. vernum*, *S. vittatum*; Imhof & Smith, 1979; Golini & Davies, 1987; Lake & Burger, 1983; Wotton, 1987). Differences in oviposition behaviour therefore do not fully explain these observations. Water discharged from the surface of Elandsdrift Dam carried less suspended material than inflowing water, and the diversion of water from Elandsdrift Dam into the neighbouring Sundays River reduced the flows downstream of the dam (Palmer & O'Keeffe, 1990). It is possible therefore that the relatively clear, and lower water levels downstream of Elandsdrift Dam may effectively simulate natural flow conditions which were present in the Great Fish River prior to the introduction of Orange River water, and so favour those taxa (such as *S. nigritarse* s.s.) whose larvae are better adapted to clear, slow-flowing conditions.

Simulium mcmahoni de Meillon
Fairly common, particularly in the Orange (Table 2) and Vaal Rivers. Absent from the Transkei, the south-western, southern and eastern Cape. Larvae prefer dead leaves, but also attach onto trailing vegetation (Roberts & Okafor, 1987) and rocks. Immatures found in slow-flowing water (0.3-1.3 m/s: Bafort et al., 1977; Roberts & Okafor, 1987). Occasionally found in impoundment tailwaters. In the Vaal River they were present throughout the year in a variety of breeding places, occasionally in high numbers (Chutter, 1967; 1968a). Females often found indoors, crawling up window panes. Often a nuisance by flying around ones legs. Eggs laid on trailing vegetation. Pest of poultry.

Simulium damnosum s.l. Theobald (complex)
This complex comprises over 40 cytologically distinct members, many of which are important disease vectors in Central and West Africa (Crosskey, 1987; Davies, 1994). In southern Africa there are at least 3 members of this complex (Crosskey, 1987), but none is known to transmit any diseases. They are one of the most common blackflies in warm, wet regions of southern Africa. Absent from the south-western Cape. Distribution extends as far south as the Tsitsikamma Forest. In the Orange River they are abundant at times (Table 2). Larvae attach onto trailing vegetation and rocks, and have a preference for fast-flowing water (around 1 m/s: Burton & McRae, 1965; Elouard, 1987). Common in the lower (turbid) reaches of rivers in the eastern Cape (Sundays, Great Fish, Keiskamma, Buffalo and Great Kei Rivers), although they are also present in clear, pristine streams, as well as polluted streams. They are typical impoundment-outlet taxa, and were often abundant downstream of Bridle Drift Dam, eastern Cape, when water was discharged from a low-level outlet. In the Vaal River, they were found in high numbers in winter where the river is wide and fringed by marginal vegetation (de Moor, 1982b). In the Tugela River, they were restricted to the middle and lower reaches (Oliff, 1960). Females feed on cattle in the Buffalo River Catchment, eastern Cape, and people in the Vaal and Orange River Catchments (pers. obs.). Members of the *S. damnosum* complex are also known to feed on fowls in Cameroon (Disney, 1972).

With the increasing growth of *Phragmites* reeds in the Orange River, and the drop in silt levels because of the dams, it is likely that *S. damnosum* s.l. will become an increasing menace. Along the Vaal River, they were estimated to cause a 30-50 % drop in milk production (Steenkamp, 1972). Males form mating swarms around the host where females seek blood (Wenk, 1981). In West and Central Africa, members of the *S. damnosum* complex transmit the nematode *Onchocerca ochengi* among cattle (Omar et al., 1979) and *O. volvulus* among people. The later disease is known as far south as central Angola (de Meillon, 1955) and near Mount Mlanji in Malawi (de Moor, pers. comm.).

Simulium adersi Pomeroy
One of the most common blackflies in southern Africa. Probably a species complex. Particularly common in polluted streams in the Transvaal highveld, but also found in pristine streams. Rare in the south-western Cape. Often abundant in the lower (turbid) reaches of rivers in the eastern Cape (Great Kei, Buffalo, Keiskamma & Great Fish Rivers). First recorded in the Orange River at Westerberg in March 1993. By April it was common in the Upington area (Table 2), and in September 1993 pupae were found at Vioolsdrift. This species either spread from the Vaal River, where it is common, or was present in the Orange River in low numbers, but not detected.

A typical impoundment-outlet species. Larvae attach onto rocks, trailing vegetation, dead leaves and other submerged vegetation, as well as sandy substrates (Crosskey, 1960). Prefers slow-flowing water. Larvae have 7 instars (Elouard, 1978), and pupate within 17 days at 20±1^{0}C (Begemann, 1980b). Tolerates high levels of ammonium (Grunewald, 1981). Mean mass of mature larvae from the Buffalo River, eastern Cape, was 0.233±0.009 mg SE (n=18; Palmer, 1991).

Females are ornithophilic (Crisp, 1956; Fallis & Raybould, 1975), but also feed on sheep, goats and humans (Gibbins, 1934; 1938; Raybould, 1965). They are attracted to hosts both visually (Begemann, 1980b) and by CO_2 (Fallis & Raybould, 1975). They are the main human-biter along the Vaal River (Begemann, 1980a), but do not bite along the Buffalo River, eastern Cape. In West Africa they transmit diseases among poultry, and have been implicated as a possible vector of human onchocerciasis (Wegesa, 1970). They have been suspected of spreading the parasitic blood sporozoan *Leucocytozoon struthio* among ostriches in the Oudtshoorn district (Hutchzermeyer pers. comm.). Adults form mating swarms near prominent markers such as trees (de Moor, 1989). Females lay about 400 eggs in clusters onto partly submerged stones (de Moor, 1989) or emergent and trailing vegetation (pers. obs.). The eggs are 202 x 135 µm in size (de Moor, 1982b), and take up to 13 days to hatch at 25^{0}C (Begemann, 1980b).

Simulium gariepense de Meillon
An extremely unusual species, and the only member of the subgenus Afrosimulium (Crosskey, 1969: 30; Car, 1983b). Males are characterised by having exceptionally large heads relative to body size, and females have an unusually long proboscis (Crosskey, 1969, 33). The larvae have unusual fan structures specialised for feeding in turbid conditions. A large-river species, endemic to southern Africa. Present in the Orange, Vaal, the Great Fish and Sundays Rivers (Chutter, 1972). Common in the Orange River in 1960 (Agnew, 1965), and directly downstream of the Hendrik Verwoerd Dam in 1992. In 1991 and the first half of 1992 it was present in the Upington area (Table 2). Thereafter it was not found, except on the 26th April 1993, when a single pupa was found at Gifkloof, and on the 16 March 1994, when a pupa was found at Prieska.

Larvae usually attach onto stones in moderately fast-flowing water. Specialised for feeding in turbid (silty) conditions. Larvae prefer slower flowing water compared to *S. chutteri*. Although reported a nuisance to cattle and people near Luckoff (de Meillon, 1953) and Colesberg (de Meillon, 1955), the shape of the female tarsal claws indicate that its preferred host is birds. It is therefore unlikely to be a major pest of livestock.

In view of the specialised habitat requirements of *S. gariepense*, and the changed conditions in the Orange/Vaal/Fish/Sundays system following impoundment, I strongly support the suggestion by Dr de Moor, of the Albany Museum, that this species be regarded a threatened species.

Simulium impukane de Meillon
Fairly common in small, temporary streams. In the Orange River it was once recorded from the tailwaters of Boegoeberg Dam (Table 2). Larvae attach onto small stones, trailing vegetation and dead leaves in slow or fast flow. Population densities seldom high. Females ornithophilic. In Tanzania they have been implicated in the spread of the sporozoan blood parasite *Leucocytozoon schoutedeni* among poultry (Fallis et al., 1973). Eggs are laid on submerged stones.

Simulium ruficorne Macquart
Very common, particularly in the drier regions of southern Africa, as well as the more polluted areas, such as the Witwatersrand. Its biology and taxonomy has been reviewed in detail by Crosskey and Büttiker (1982). Seldom found in the main channel of the Orange River (Table 2), but very common in small mineralised streams and agricultural seepage waters throughout the catchment. It is one of the first species to colonize new habitats. Larvae were found within a week after flow resumed in

a stream in Zimbabwe (Harrison, 1966), and within 24 days in the Great Fish River (Chutter, 1972). Larvae attach onto dead leaves, trailing vegetation, rocks or algae. They are usually found in slow-flowing water (including stagnant pools), although they have also been recorded in current speeds of up to 1.4 m/s (Fain & Elsen, 1973). Tolerate high water temperatures and poor water quality, although they are also found in pristine streams. In the Buffalo River, eastern Cape, they were encountered where industrial and domestic pollution enters the river. Females are anautogenous (Takaoka, 1988), and feed on birds including poultry (Orian, 1962, in Crosskey & Büttiker, 1982) and francolin (Freeman & de Meillon, 1953: 99). A pest of poultry and sparrows on the island of Mauritius, where it is suspected of transmitting fowl pox (Orian, 1962, in Crosskey & Büttiker, 1982). Eggs are laid on trailing vegetation or stones.

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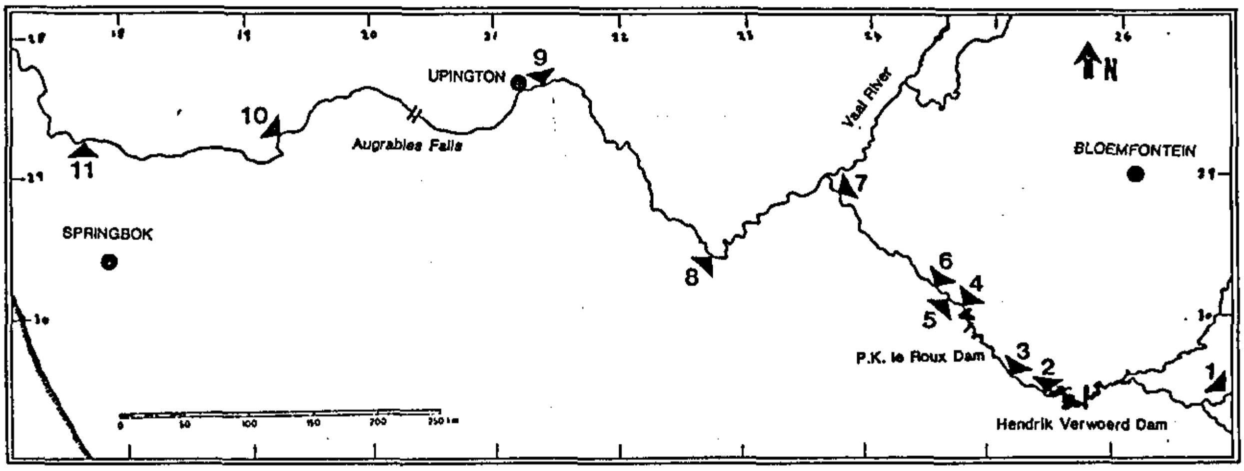
3. STUDY AREA

The blackfly problem along the Orange River stretches from the Hendrik Verwoerd Dam to beyond Vioolsdrift, a distance of over 1000 km (Fig. 1 & 4). Between the Hendrik Verwoerd and P. K. Le Roux Dams the problem can be controlled by flow manipulation, and this part of the river was therefore largely omitted from this project. Two aerial surveys of the river between Hopetown and Augrabies Falls identified between 98 (at high flow) and 134 (at low flow) treatable rapids and riffles.



1=Walaza; 2=2.5 km downstream of Hendrik Verwoerd Dam; 3=Serfontein Bridge; 4=0.5 km downstream of P.K. le Roux Dam; 5=Havenga Bridge; 6=Orania; 7=Marksdrift; 8=Prieska; 9=Gifkloof; 10=Onseepkans; 11=Vioolsdrift.

Figure 4. Map of the Orange River showing sampling sites and major impoundments and towns.

The river between P.K. le Roux Dam and Upington consists primarily of a single channel, ranging in width from 50 m to over 300 m. This section of the river is characterised by long stretches of slow-flowing water, interspersed by rapids. The average gradient over this distance is 0.49 m/km, and the number of rapids per 10 km ranged from 0 to 3. Between Upington and Augrabies Falls the river splits into several channels, and is up to 3.2 km wide from bank to bank (Fig. 6 & 7). Average gradient in this part of the river is 1.49 m/km. Alluvial

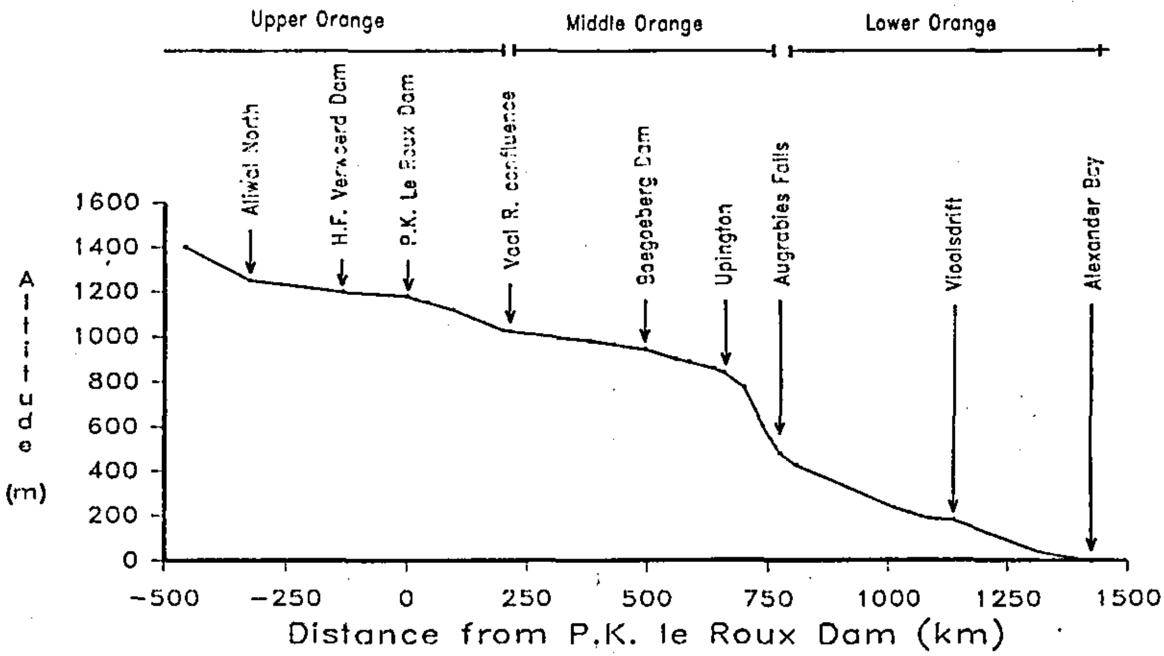


Figure 5. Profile of the middle and lower Orange River.

sediments in this part of the river are intensively farmed. At Augrabies Falls the river drops 56 m and continues through a narrow gorge for some 18 km before opening out at Blouputs. From Blouputs to Vioolsdrift the river is highly braided, and provides nearly continuous blackfly breeding sites. Average gradient in the lower reaches is 0.75 m/km.

Sites sampled

Eleven sites between Walaza (upstream of the Hendrik Verwoerd Dam) and Vioolsdrift were sampled on two occasions (summer and winter) in 1992. Specifications of these sites are provided in Appendix A. Specifications of major impoundments are provided in Appendix B.

Trials of the downstream "carry" of larvicides were undertaken at six localities in the vicinity of Upington (Table 4; Fig. 6). Gradient in the six experimental zones ranged from 0.13 m/km at Grootdrink, to 2.39 m/km at Neus (measured over 10 km intervals from 1:500 scale maps). The stream bed consisted largely of granite boulders and sandbanks. Rapids were interspersed by long stretches of pool, about 1-2 m deep.

Detailed sampling of river conditions and invertebrates occurred throughout the study period at various sites downstream of Gifkloof Weir (Fig. 8). Gifkloof was chosen because of its proximity to Upington (20 km upstream), its limited access (the weir belongs to the Department of Water Affairs), and rubble left from construction of the weir provided substrates which could be sampled under a wide variety of flows. However, as river levels dropped, the sampling site was changed from G2 to G3, and later to G4 (Fig. 8).

Aquatic vegetation

The most abundant vegetation along the middle and lower reaches of the Orange River were *Phragmites* spp. reeds, and to a lesser extent, the reed *Arundo donax*. Stems and roots of these reeds trailing in the current formed important attachment sites for blackfly larvae. The most abundant algae attached to the stones-in-current were the pollution-tolerant *Cladophora glomerata* and *Stigeoclonium tenue*. Other taxa which were common at times were *Spirogyra* sp. and the blue-green algae *Nostochopsis* sp.. The moss *Fontinalis antipyretica* was found in fast-flowing water a few times. In slow-flowing water, water hornwort (*Ceratophyllum demersum*) and fennel-leaved pondweed (*Potomageton pectinatus*) formed dense stands. The willow-herb (*Ludwigia* sp.) formed dense stands in shallow water at the edges. Water Fern (*Azolla filiculoides*) grew on the surface of quiet backwaters.

Table 4. Details of sites in the Orange River in the vicinity of Upington, where larvicide "carry" trials were undertaken.

Site	Grid		Channel		Gradient
	South	East	Width (m)	Type	(m/km)
Sishen Bridge	28°46'	21°52'	280	Single	0.55
Grootdrink	28°33'	21°45'	190	Single	0.13
Gifkloof Weir	28°26'	21°23'	380	Single/Braided	1.14
Upington Bridge	28°27'	21°15'	250	Single/Braided	1.00
Kanoneiland	28°38'	21°05'	160 + 140	Braided	1.00
Neus	28°46'	20°43'	100 + 150	Braided/Single	2.39

"Width" refers to the approximate bank-full width. "Gradient" refers to the drop in altitude over the first 10 kilometres from the point of application.

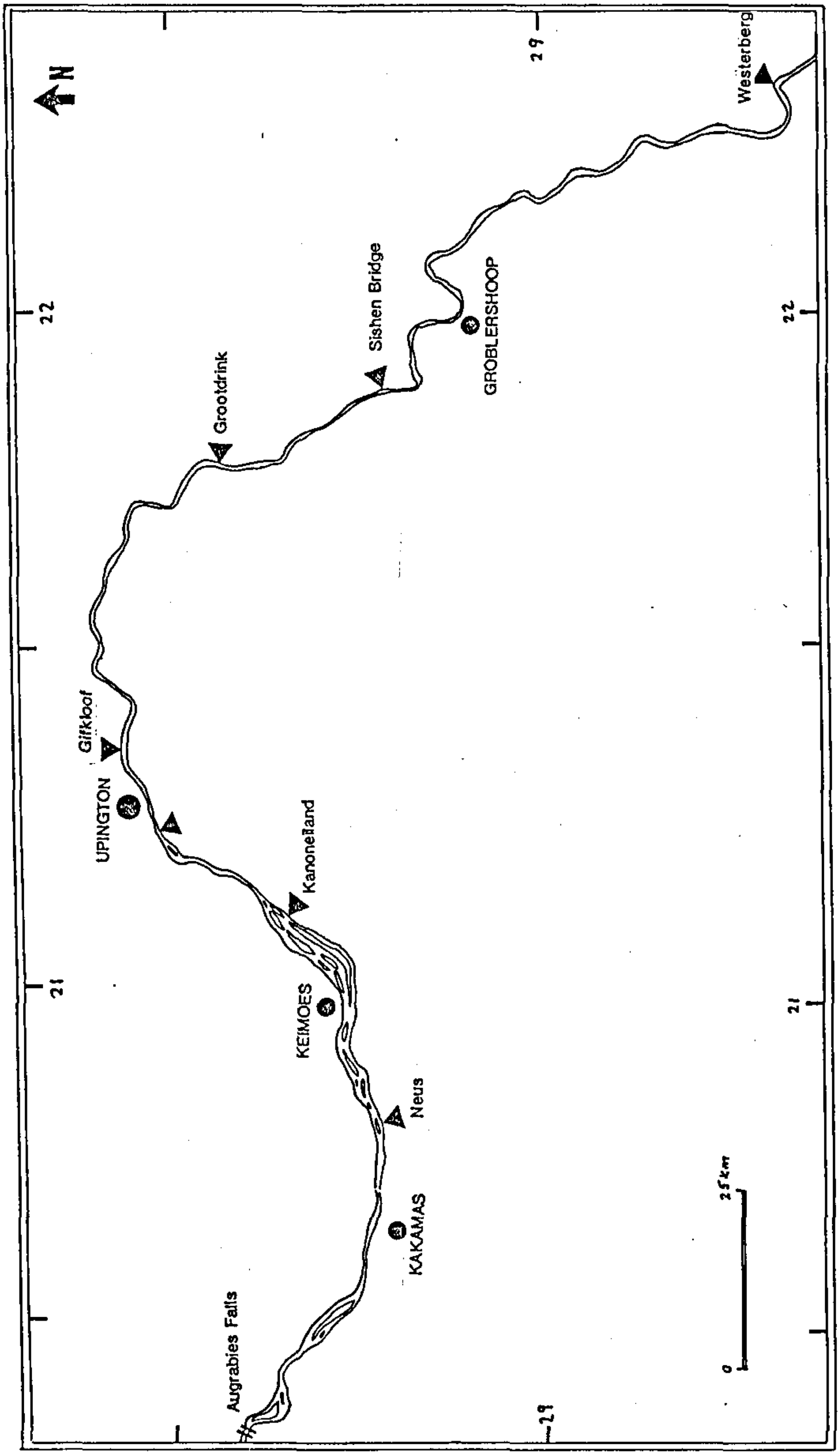


Figure 6. Map of the Orange River in the vicinity of Upington, showing sites where "carry" trials were undertaken. Notice the multiple channels downstream of Upington.

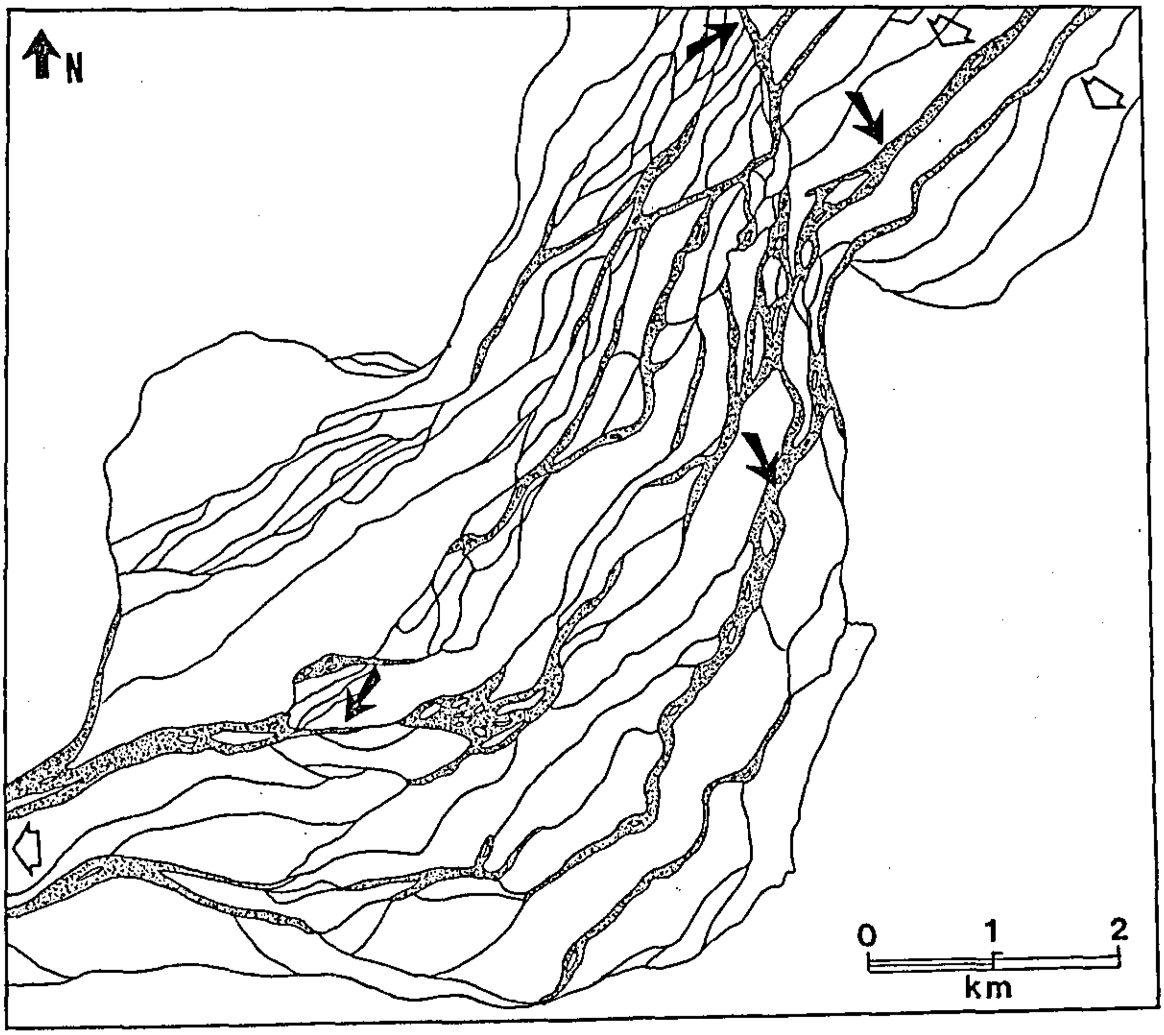


Figure 7. Map of the Orange River between Kanoneiland and Keimoes. The main channel is shaded. Solid arrows indicate application points.

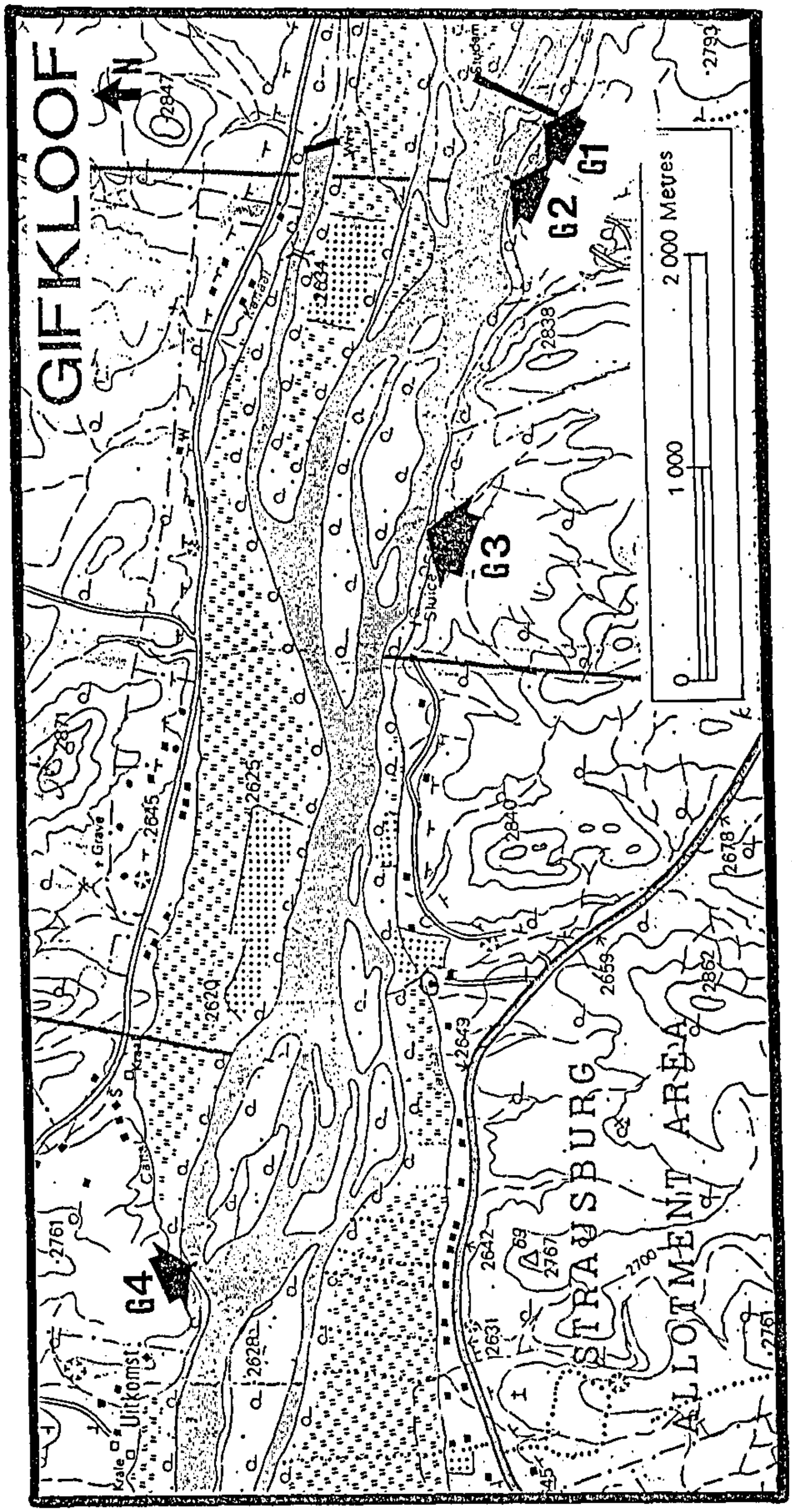


Figure 8. Map of the Orange River at Gifkloof, showing the sites (G1 to G4) which were sampled regularly.

4. RIVER CONDITIONS

4.1 Flow

Accurate flow determination is important for blackfly control because, among other things, flow determines the amount of larvicide required, as well as the distance between successive applications.

Daily flow data for the Orange River were supplied by the Department of Water Affairs. Reliable gauging weirs were situated at P.K. le Roux Dam (D3R003-M01), Marksdrift (D3H008), Prieska Bridge (D7H002), Boegoeberg Dam (D7H008) and Vioolsdrift (D8H003). In addition, flow was measured by the author at an old weir at Kanoneiland and a new weir at Neus (between Keimoes and Kakamas).

Frequency analysis of daily average flows recorded at Boegoeberg Dam before and after major impoundment shows that median flows have not changed since impoundment, but that both low and high flows (except for major floods), have become less frequent (Fig. 9). Five categories of flows were recognised, ranging from very low (<16 m^3/s) to very high (>670 m^3/s)(Fig. 9). The categories were based on the probability of exceedance at the 10th, 40th, 60th and 90th percentiles before the impoundments were built (Fig. 9).

One of the main effects of impoundments in the Orange River has been to convert the river from seasonal to perennial by reducing downstream seasonal flow-fluctuations (Fig. 10). Summer flows are now significantly lower than before, and winter flows are much higher (Fig. 11). The higher winter flow has favoured the breeding of *S. chutteri* at the expense of other blackfly species. However, short-term (twice-daily) flow-fluctuations have been increased because of generation of hydro-electric power. Each turbine requires 170 m^3/s of water, and for most of the year (excluding periods of drought), at least one turbine is switched on between 6h00 and 10h00, and again between 16h00 and 20h00 (Groenewald pers. comm.). The minimum release for the rest of the day is 80 m^3/s (Groenewald pers. comm.). Data on hourly flow-fluctuations are not available for sites upstream of Prieska, but it is clear from the macroinvertebrate assemblages that the effects of hourly flow-pulses do not extend further than Marksdrift (200 km downstream of P.K. le Roux Dam).

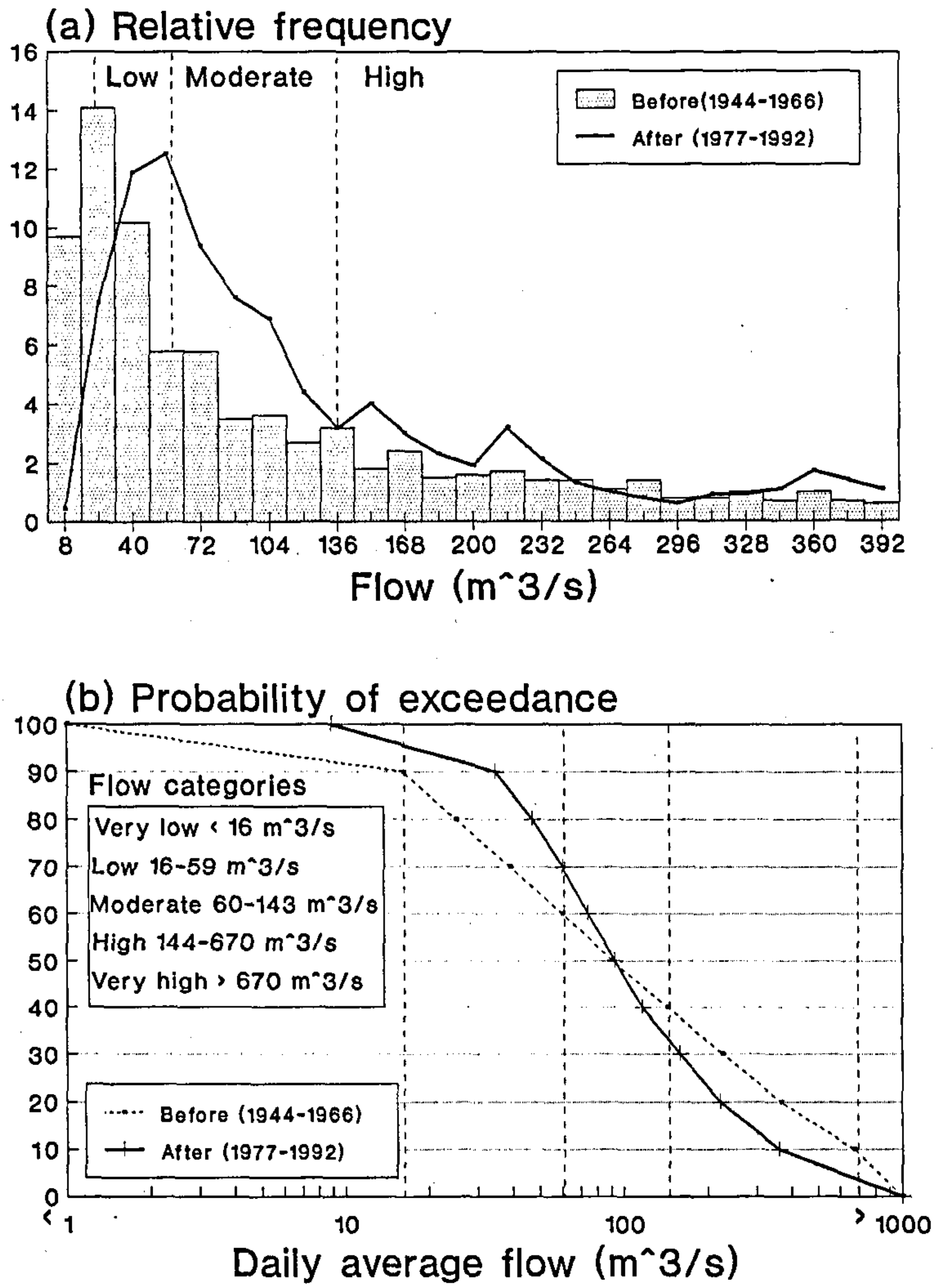


Figure 9. Frequency analysis (a) and the probability of exceedance (b) of the daily average flows at Boegoeberg Dam (D7H008) before and after major impoundment. Vertical dashes demarcate boundaries between flow categories. [Data: DWA, Pretoria.]

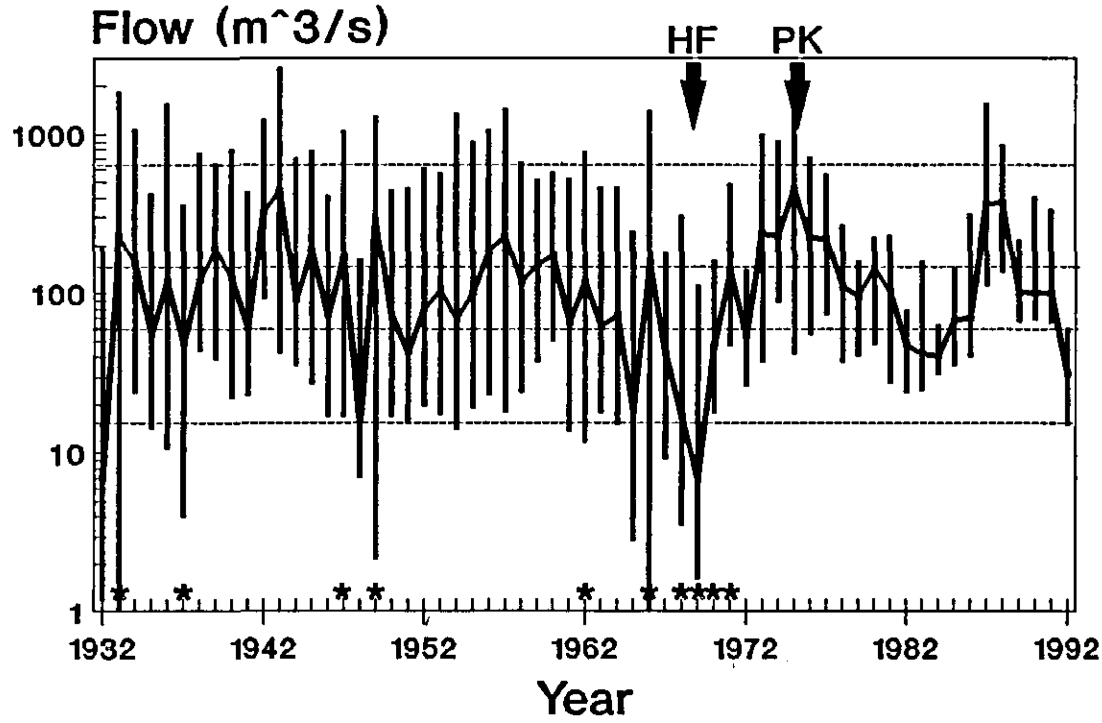


Figure 10. Median annual flows at Boegoeberg Dam (D7H008) measured daily between 1932 and 1992. Bars indicate the 10th and 90th percentiles. Stars indicate times the river was dry. Arrows indicate time of completion of impoundments. [Data: DWA, Pretoria.]

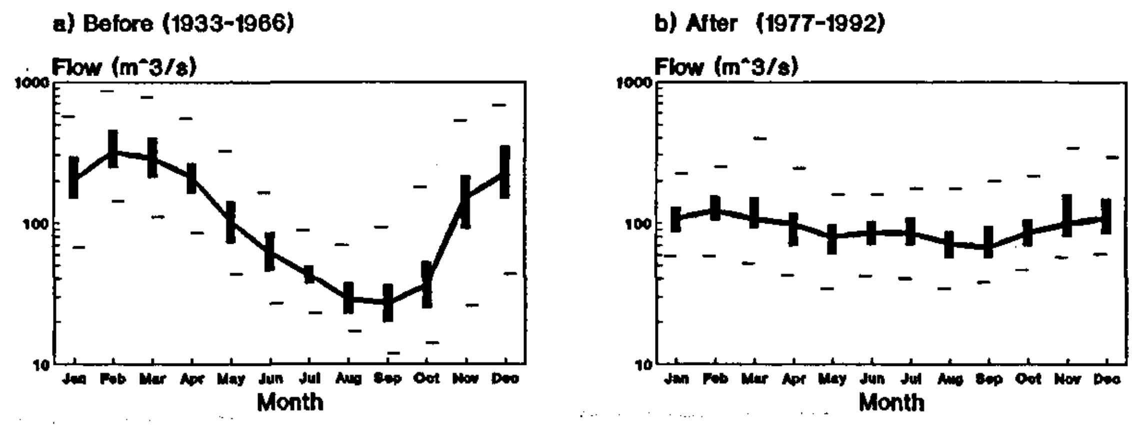


Figure 11. Median monthly flows at Boegoeberg Dam (D7H008) measured daily before and after major impoundment. Bars indicate the 40th and 60th percentiles, and horizontal lines indicate the 20th and 80th percentiles. [Data: DWA, Pretoria.]

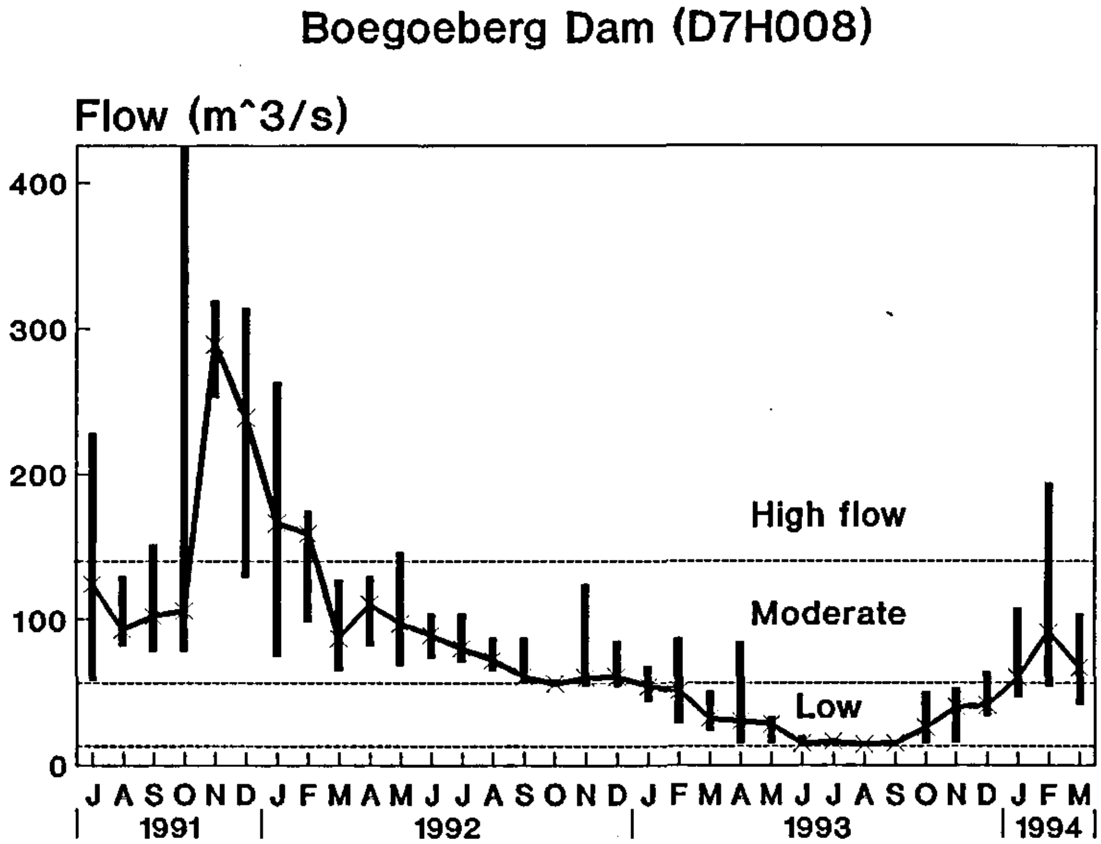


Figure 12. Median, minimum and maximum monthly flow at Boegoeberg Dam (D7H008) during the study period. Horizontal lines demarcate boundaries between flow categories. [Data: DWA, Pretoria].

In 1991 flow in the Orange River was variable, with high flow (>143 m^3/s) in March and again in November (Fig. 12). Towards the end of 1991, discharge from P.K. le Roux Dam exceeded 150 m^3/s for 60 days, a condition which was likely to have led to an outbreak of blackflies had the river not been treated with larvicides.

In 1992 flow dropped steadily throughout the year and continued to drop in 1993, reaching a record low in September 1993 (Fig. 12). Daily average releases from P. K. le Roux Dam in September 1993 ranged between 40 and 55 m^3/s, of which roughly 15 m^3/s (or 33 %) reached Boegoeberg Dam, and roughly 3 m^3/s (or 6 %) reached Vioolsdrift. Accurate flow determination became increasingly difficult at low flow (<60 m^3/s) because of abstraction, evaporation and agricultural return flows. The amount of water returned to the river depended on how much was used by farmers, and fluctuated hourly. The recently completed weir at Neus diverted 98 % of the river's flow into the Kakamas canal system, leaving 0.2 m^3/s in the river as compensation. Water from the Kakamas canals was returned to the river downstream of Kakamas, and flow at Augrabies Falls was estimated at 5 to 7 m^3/s. The stretch of river between Neus and Kakamas was therefore not treated for blackfly control on the 26th September 1993. These changes in flow (both spatially and temporally) emphasise the importance of good and frequent communication between the Blackfly Control Programme and the Department of Water Affairs.

4.2 Water temperature

Water temperature dictates the rate of blackfly larval development, and is therefore an important consideration when determining the time interval between successive larvicide applications (see Chapter 5).

Water temperature also affects the efficacy of larvicides, and may determine the choice and dosage of a particular larvicide. The efficacy of both temephos and *B.t.i.* is reduced in cold water. Temephos has been shown to have no effect at 7-9°C (Fredeen, 1987), and little effect at temperatures less than 18°C (Back et al., 1979; Rodrigeus & Kaushik, 1984). For this reason temephos is not regularly used for blackfly control in Canada (Dankwa pers. comm.). Although *B.t.i.* works over a wider range of temperatures than temephos, mortality due to *B.t.i.* applied at 20°C was twice (80,6 %) that recorded at 10°C (40.4 %) (Molloy et al., 1981). Cold temperatures therefore require increased dosages (Lacoursiere & Charpentier, 1988). In the Orange River however, winter applications (at 12°C) of both temephos and *B.t.i.* were effective against blackfly larvae, and increased dosages did not appear necessary.

Spot temperatures were measured at various localities in the Orange River with a mercury thermometer, and maximum-minimum temperatures were measured weekly with a mercury maximum-minimum thermometer placed in slow-flowing water at Gifkloof. Water temperatures at Gifkloof ranged from 8°C (in July 1992) to 29°C (in January/February)(Fig. 13). Weekly temperatures usually fluctuated by 2-3°C. The highest recorded weekly fluctuation occurred in the second week of April 1993, when Boegoeberg Dam (143 km upstream) was drained: Temperatures dropped from 23 to 15 °C (Fig. 13). Although temperatures were reasonably consistent from year to year, winter (July) temperatures in 1993 were significantly warmer than in the previous two years. Total annual degree days were about 7150.

Longitudinal changes in temperature were of concern because warmer water in the lower reaches of the river was likely to increase larval development rates, so that different reaches of the river would require treatments at different times. In the absence of temperature data, head-capsule lengths of final

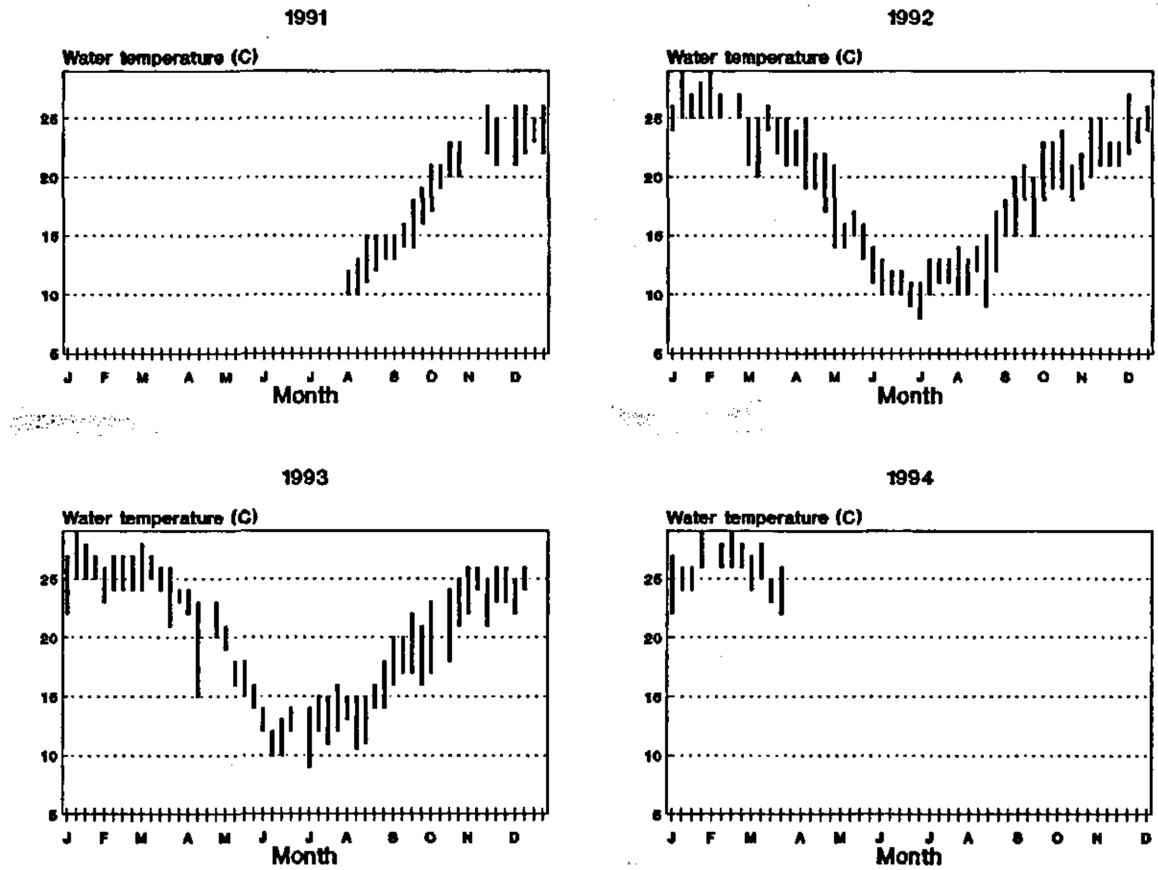


Figure 13. Maximum and minimum water temperatures measured weekly at Gifkloof.

instar *S. chutteri* larvae were used as "biological" thermometers. Head-capsules lengths of larvae collected monthly at Gifkloof were used to calibrate the "thermometer" (Fig. 14 & 15). Head capsule lengths of final instar larvae collected from Serfontein Bridge (upstream of P.K. Le Roux Dam) in summer (15-18th February 1992) were significantly longer than (x=360 µm ±22SD, n=40) than those collected downstream of P.K. Le Roux Dam (x=348 µm ±21SD, n=137; Student's t-test; t=31, $p<0.03$), indicating warmer temperatures downstream of the dam (Fig. 16; Table 5). There was no significant difference between head-capsule lengths of larvae collected at three sites downstream of P.K. Le Roux Dam (Student's t-test; $P>0.8$), indicating similar water temperatures at these sites at this time of the year (Fig. 16). In winter (13-22nd July 1992), larvae from Onseepkans (x=401 µm ±22, n=40) and Vioolsdrift (x=404 µm ±18SD, n=37) were significantly smaller than larvae collected at Gifkloof (x=437 µm ±21, n=40) upstream of the Augrabies Falls (Fig. 16; Table 5). Larvae collected from Orania were slightly smaller (x=421 µm ±23, n=12) than those collected from Prieska (x=435 µm ±21, n=28) and Gifkloof (x=437 µm ±21, n=40; Fig. 16). This means that larval development downstream of Augrabies Falls in winter is faster than upstream, and more applications would be required to adequately treat the river downstream of Augrabies compared to upstream.

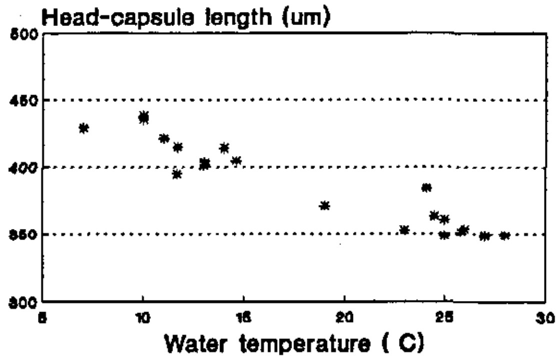


Figure 14. Average head-capsule lengths of final instar *S. chutteri* larvae as a function of spot water temperature at the time of collection.

Head-capsule lengths of larvae collected up- and downstream of the Hendrik Verwoerd and P.K. le Roux Dams in winter show how the dams have altered river temperatures (Fig. 16). A study by Allanson and Jackson (1983: 8) showed that water at the bottom P.K. le Roux Dam (>25 m depth) is always cold (10-14^{0}C), whereas the temperature of surface-released water varies from <14^{0}C, when mixed, to 20-22^{0}C, during stratification. Thermal stratification of P.K. le Roux Dam begins in October/November, and lasts until May (Allanson & Jackson, 1983: 8). Maximum-minimum thermometers placed downstream of P.K. le Roux Dam in April 1992 showed that river temperatures at this time of the year (during which average daily flow was between 87 and 130 m^3/s) were only slightly affected by P.K. le Roux Dam. Likewise, spot water temperatures measured in February 1992 were similar directly downstream of the dam (25.8^{0}C) to further downstream (22.9-27.6^{0}C). However, spot water temperature directly downstream of Hendrik Verwoerd Dam in February 1992 was cold (18.9^{0}C), but equilibrated (to 24.9^{0}C) within 42 km downstream (Table 5). Studies of the thermal recovery of rivers elsewhere indicate that at discharges between 50 and 200 m^3/s, temperatures re-equilibrate after 130 to 180 km (Palmer & O'Keeffe, 1989).

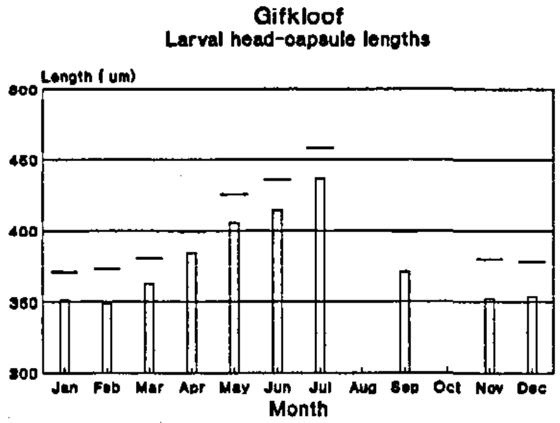


Figure 15. Average head-capsule lengths (±1SD) of final instar *S. chutteri* larvae collected monthly at Gifkloof.

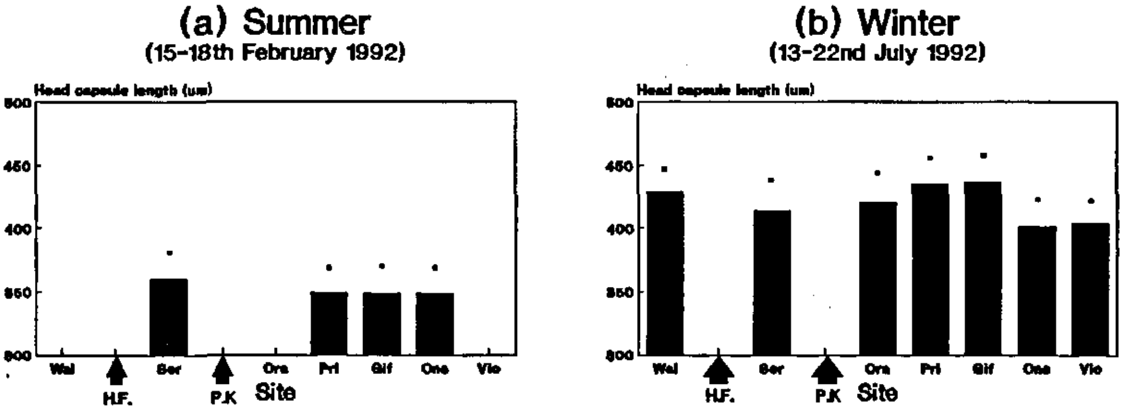


Figure 16. Average head-capsule lengths of final instar *S. chutteri* larvae collected at various sites along the Orange River in summer (a) and winter (b). One SD is indicated.

Table 5. Conditions measured at various sites in the Orange River in summer (15-18th Feb. 1992) and winter (13-22nd Jul. 1992).

Site	Spot temp (°C)		TSS (mg/l)		Benthic algae		Larval abundance		Head-capsule lengths (µm)	
	S	W	S	W	S	W	S	W	S	W
Walaza	22	7	278	12	0	2	1	3	-	429±18(29)
- - - - - - Verwoerd										
H.F. Verwoerd Dam (2.5 km)	19	11	83	27	1	2	2	2	-	-
Serfontein Bridge	25	14	56	32	2	2	2	2	360±21(40)	414±24(40)
- - - - - - P.K. Le Roux										
P.K. Le Roux Dam (0.5 km)	26	11	35	36	0	2	0	0	-	-
Havenga Bridge	25	9	23	26	0	3	0	2	-	-
Orania	23	11	43	38	1	3	1	2	-	421±23(12)
Marksdrift	24	11	39	36	1	3	2	1	-	-
Prieska	28	10	58	32	2	3	2	3	349±20(44)	435±21(28)
Gifkloof	27	10	55	31	2	3	3	3	348±22(50)	437±21(40)
Onseepkans	25	13	55	36	3	3	2	3	348±21(43)	401±22(40)
Vioolsdrift	27	13	49	36	2	3	1	3	-	404±18(37)

Benthic algae and blackfly larval abundance were ranked on a 4-point scale (0=none; 1=present; 2=common; 3=abundant). Head-capsule lengths were of final instar *S. chutteri* larvae are presented as averages ± 1 Standard deviation. (Sample size is indicated in brackets.) Dotted lines indicate positions of impoundments.

4.3 Total suspended solids (TSS) and planktonic algae

The concentration of TSS (silts, clays and phytoplankton) has important implications in the choice of larvicides. Temephos is suited to high TSS because it is more effective when adsorbed onto particles. *B.t.i.* on the other hand is suited to low TSS because it "competes" with TSS for ingestion by blackfly larvae (Guillet, et al., 1985b). Silts and clays are good bacterial adsorbents, and as much as 99.8 % of *B.t.i.* may be found in the mud within 45 minutes after application (Ohana et al., 1987). Ignoffo et al. (1981, in Gaugler & Finney 1982) found that water with as little as 20 mg/l caused a marked reduction in the efficacy of *B.t.i.* against mosquitoes. Ramoska et al. (1982) found that clay particles reduced the efficacy of *B.t.i.* against blackflies when TSS exceeded 500 mg/l. Blackfly ingestion rates increase asymptotically with increasing food availability, levelling off at about 100 mg/l (Gaugler & Molloy, 1980; Hart & Latta, 1986; Palmer, 1991). Work in West Africa has found that *B.t.i.* no longer works effectively when turbidity exceeds 150 JTU (roughly 150 mg/l) (Kurtak pers. comm.). This value of 150 mg/l TSS has been adopted in the present project as the threshold value above which temephos should be used in preference to *B.t.i.*. Experience in West Africa has also found that *B.t.i.* no longer works effectively when the concentration of planktonic algae exceeds 1 500 cells per ml (Kurtak pers. comm.). This is equivalent to a chlorophyll a concentration of roughly 4 µg/l (Young, 1986). Typical values for chlorophyll a measured near the P. K. le Roux Dam outlet in between 1977 and 1983 were 1-4 µg/l, although values of up to 20 µg/l were measured in March 1983 (Hart et al., 1983). Algal blooms in lake P. K. le Roux were restricted to summer months, and changed from high densities of *Anabaena circinalis* in December, to high densities of *Microcystis aeriginosa* in February to March (Hart et al., 1983).

The concentration of TSS was measured weekly at Gifkloof by filtering 200 to 800 ml (depending on clogging) of river water through pre-weighed Whatman® GF/C filters. Filters were dried for at least 24 hrs at 60°C, and weighed on a Masskof® microbalance accurate to 0,0001 g. The abundance of planktonic algae was also determined weekly by filtering 200 ml of river water through Whatman® GF/C filters. Filters were placed on glass slides containing a few drops of Golden® [Cane-sugar] Syrup, and examined under a compound microscope at 400 x magnification. The method was crude because different sized cells scored the same. Filamentous algae were counted in "units" which were 7 times the width of the filament, again a method developed and used by the OCP (Kurtak, pers. comm.).

The concentrations of TSS at Gifkloof between July 1991 and March 1994 ranged from 5 to 144 mg/l (Fig. 17; Appendix C). This is well below the threshold concentration above which *B.t.i.* no longer works effectively.

In June 1993 it was realised that Secchi depth values would be a more practical measure of suspended particles for the control programme. Work conducted at the P.K. Le Roux Dam by Hart (1988), together with limited data collected in the present project, provided a relationship between TSS and Secchi depths readings (Fig. 18). The threshold value of 150 mg/l TSS approximates a Secchi depth of about 12 cm (Fig. 18). In other words, if the Secchi depth exceeds 12 cm, *B.t.i.* should be effective at the recommended dosage.

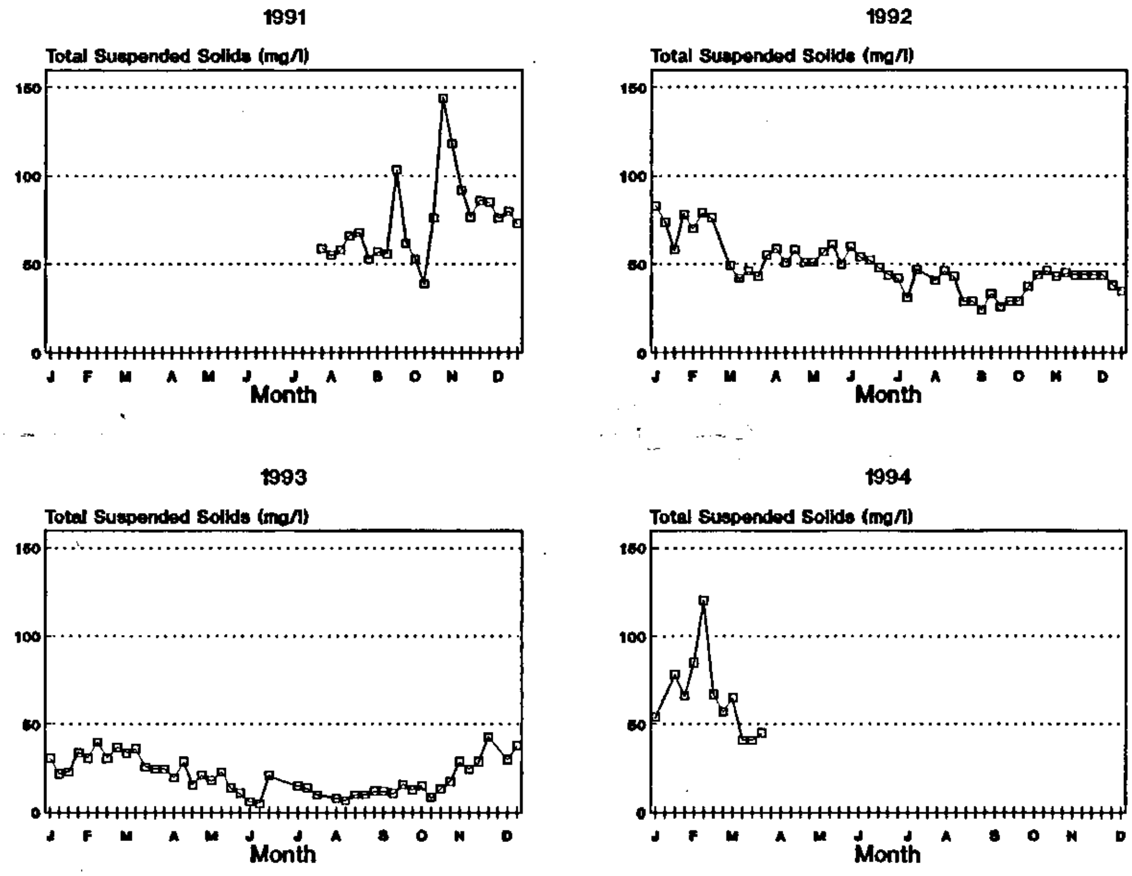


Figure 17. Weekly changes in the concentration of total suspended solids at Gifkloof between July 1991 and March 1994.

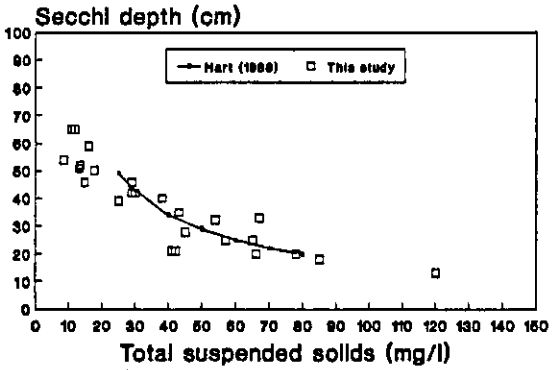


Figure 18. Secchi depth transparency in relation to the concentration of total suspended solids in the Orange River.

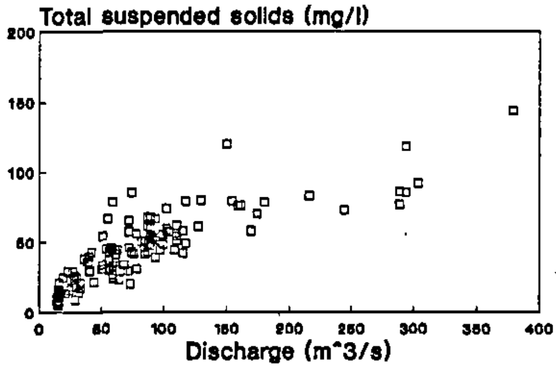


Figure 19. The concentration of total suspended solids at Gifkloof, as a function of flow at Boegoeberg Dam (D7H008), measured between July 1991 and March 1994.

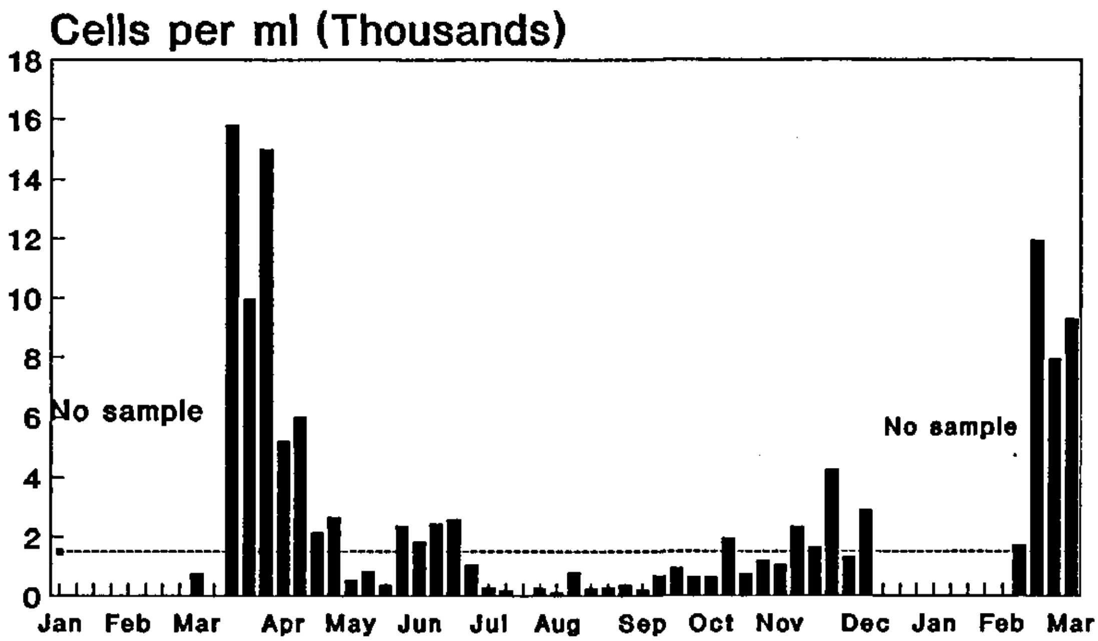


Figure 20. Weekly changes in planktonic algal abundance at Gifkloof between March 1993 and March 1994. The dotted line demarcates the threshold value above which *B.t.i.* is considered ineffective (Kurtak pers. comm.).

The river became increasingly clear as the flow dropped, and Secchi depths of up to 65 cm were recorded (Figs. 18 & 19). Blackfly larval abundance showed no relationship with the concentration of TSS, although *S. chutteri* were replaced by other species when the TSS dropped below about 20 mg/l. In March 1993 there was a *Microcystis* sp. and *Anabaena* sp. bloom (Fig. 20; Table 6), and under these conditions, blackfly larvae were almost completely absent (Fig. 27). Studies elsewhere have found that *Microcystis* spp. and *Anabaena* spp. metabolites are highly toxic (Marten, 1986; Harding, pers. comm.). It is possible therefore that autumn algal blooms in the Orange River are a natural biocontrol agent for blackfly larvae.

A "carry" trial conducted during the algal bloom confirmed that *B.t.i.* does not work effectively under these conditions (Trial 34, Table 12). Algal blooms do not occur every year, but when they do they occur between December and March (Hart et al., 1983). Limited data collected in this study indicates that blooms are unlikely to occur if the TSS exceeds 40 mg/l (Fig. 21).

The large-scale river surveys conducted in February and July 1992 showed the stabilising effects of the P.K le Roux and Hendrik Verwoerd Dams on suspended solids (Table 5). The concentration of total suspended solids at Walaza (upstream of Hendrik Verwoerd Dam) ranged from 12 to 278 mg/l, whereas at Havenga Bridge (downstream of P.K. Le Roux Dam), the concentration ranged from 23 to 26 mg/l (Table 5).

Violent summer thunderstorms in the middle and lower reaches often contributed localised inputs of highly turbid water. For example, in March 1994, the Hartbees River, near Kakamas, carried a TSS concentration of 2775 mg/l, and increased TSS levels in the Orange River at Blouputs (54 km downstream of the confluence with the Hartbees River) from 65 to 100 mg/l.

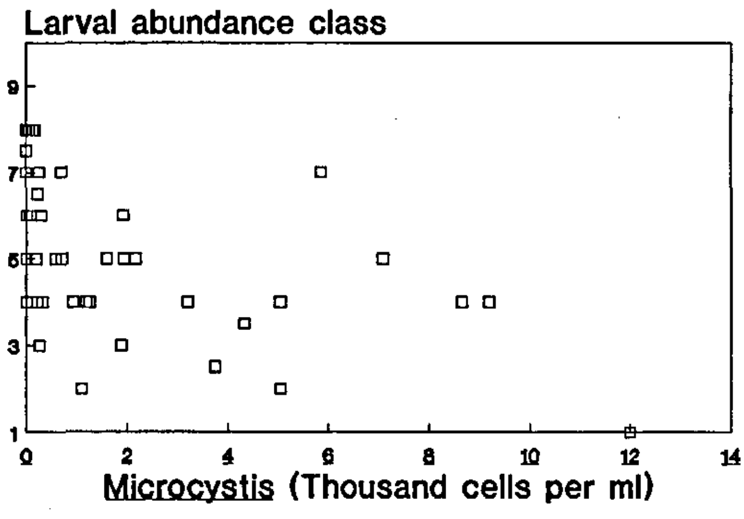


Figure 21. Median abundance of blackfly larvae as a function of the abundance of *Microcystis* sp. at Gifkloof. Blackfly abundance was ranked according to a 10 point scale shown in Figure 23.

Table 6. Abundance of planktonic algae in the Orange River, measured weekly at Gifkloof. Data are presented as cells/ml, with percentage contribution in brackets.

Date	*Microcystis*	Filamentous	Diatoms	*Scenedesmus*	*Merismopedia*	*Pediastrum*	Other	TOTAL
1 Mar 93	621(80)	156(20)	0	0	0	0	0	777
8 Mar 93	-	-	-	-	-	-	-	-
15 Mar 93	8640(54)	7040(44)	0	0	0	0	128(1)	16000
22 Mar 93	5040(50)	4700(47)	0	0	0	0	200(2)	10000
29 Mar 93	12060(80)	2880(19)	0	0	0	0	45(<1)	15000
5 Apr 93	3754(72)	1170(22)	0	0	0	0	265(5)	5200
12 Apr 93	5034(83)	150(2)	0	0	0	0	804(13)	6000
19 Apr 93	1911(89)	21(1)	0	0	0	0	214(10)	2140
26 Apr 93	1878(71)	0	8(<1)	148(6)	148(6)	206(8)	249(9)	2650
3 May 93	256(45)	0	57(10)	171(30)	0	0	85(15)	570
10 May 93	315(37)	18(2)	132(16)	162(19)	0	0	209(25)	840
17 May 93	0	0	28(8)	66(18)	0	0	279(74)	370
24 May 93	1189(59)	456(23)	93(5)	95(5)	0	0	180(9)	2020
31 May 93	1253(69)	131(7)	84(5)	84(5)	0	0	262(14)	1820
7 Jun 93	1934(79)	122(5)	66(3)	85(4)	0	0	224(9)	2400
14 Jun 93	1588(61)	504(19)	26(1)	54(2)	0	0	416(16)	2600
21 Jun 93	626(59)	69(7)	69(7)	78(8)	0	0	175(18)	1050
28 Jun 93	-	-	-	-	-	-	-	-
5 Jul 93	0	18(6)	84(29)	0	0	0	186(64)	290
12 Jul 93	0	0	57(28)	0	0	0	142(71)	200
19 Jul 93	0	0	0	0	0	0	28(100)	28
26 Jul 93	0	79(28)	60(21)	14(5)	0	0	131(46)	285
2 Aug 93	0	36(28)	32(25)	23(18)	0	0	36(28)	130
9 Aug 93	285(42)	42(6)	75(11)	89(13)	0	0	179(26)	680
16 Aug 93	128(48)	9(4)	47(18)	33(13)	0	0	47(18)	270
23 Aug 93	0	18(6)	85(28)	112(37)	0	0	85(28)	300
30 Aug 93	52(14)	5(1)	133(35)	95(25)	0	0	95(25)	380
6 Sep 93	0	5(2)	76(34)	66(30)	0	0	76(34)	220
13 Sep 93	0	4(1)	147(21)	194(28)	0	103(15)	213(35)	690
20 Sep 93	171(18)	0	66(7)	57(6)	0	214(22)	441(46)	950
27 Sep 93	251(37)	9(1)	122(18)	75(11)	0	101(15)	108(16)	680
4 Oct 93	0	5(1)	187(28)	267(40)	0	57(9)	141(21)	670
11 Oct 93	919(47)	17(1)	164(8)	261(13)	141(7)	171(9)	278(14)	1960
18 Oct 93	23(3)	0	199(26)	123(16)	151(20)	66(9)	189(25)	755
25 Oct 93	71(6)	8(1)	260(22)	289(24)	74(6)	71(6)	453(37)	1206
1 Nov 93	212(20)	0	137(13)	90(8)	99(9)	246(23)	270(25)	1060
8 Nov 93	0	0	232(16)	227(15)	189(13)	288(19)	545(37)	1490
15 Nov 93	189(11)	8(<1)	184(11)	169(10)	302(18)	302(18)	497(30)	1660
22 Nov 93	924(44)	27(1)	208(10)	193(9)	170(8)	94(4)	359(17)	2100
29 Nov 93	212(16)	4(<1)	213(16)	188(14)	266(20)	218(16)	222(17)	1330
6 Dec 93	701(24)	0	348(12)	507(17)	171(6)	754(26)	403(14)	2900
14 Feb 94	1101(63)	0	132(8)	147(8)	0	0	365(21)	1750
21 Feb 94	9189(76)	36(<1)	675(6)	84(1)	0	0	2038(17)	12060
28 Feb 94	5849(73)	16(<1)	24(3)	200(2)	0	32(<1)	1635(20)	8010
7 Mar 94	7082(76)	28(<1)	28(3)	28(3)	47(5)	0	1165(12)	9319
14 Mar 94	4139(85)	0	25(5)	15(3)	0	0	31(6)	5080
21 Mar 94	2160(83)	18(1)	21(8)	0	0	0	21(8)	2600
28 Mar 94	0	0	190(16)	451(38)	0	0	546(46)	1190

4.4 Benthic algae

Benthic algae provide insight into river conditions, and are supposedly important to blackfly larvae because they make substrates less suitable for attachment (Wheeler & Hershey, 1994). I am not convinced about this because high numbers of blackfly larvae were often found attached to algae. Benthic algae did, however, provide habitat for the larvae of the midge *Cardiocladius ?africanus* (an occasional predator of small blackfly larvae), and the muscid fly, Limnophorinae (also a predator of blackfly larvae). Benthic algae may therefore play an important role in blackfly population dynamics.

The abundance of benthic algae was assessed weekly at Gifkloof, as well as at various sites along the river in the summer (February) and winter (July) of 1992. Initially, assessment was based on a 4-point scale [1=none; 2=present; 3=common; 4=abundant], but in April 1993, assessment was changed to a percentage cover, because it was more accurate. No distinction was made between the different species of algae, although the most common species were *Cladophora glomerata* and *Stigeoclonium tenue*, both of which are well-established indicators of polluted water (Dell'uomo, 1991). *Cladophora glomerata* is toxic to mosquito larvae (La Londe et al., 1979), and may well be toxic to blackfly larvae, although this has not yet been examined.

The abundance of benthic algae fluctuated during the study period from next to nothing in the autumn (April/May) to an almost complete cover in late winter (Fig. 22). In March 1992, the turbidity dropped (Fig. 17), and the benthic algae showed a corresponding increase, presumably because of increased light penetration (Fig. 22).

Benthic algae were absent from Walaza (upstream of the Hendrik Verwoerd dam) in February 1992, presumably because of reduced light penetration and the scouring action of high TSS (278 mg/l)(Table 5). Benthic algae were also absent for at least 18.5 km downstream of P.K. le Roux Dam (to Havenga Bridge), presumably because of the daily flow-fluctuations resulting from the generation of hydro-electricity (Table 5). At Orania (44.9 km downstream of the P. K. le Roux Dam), benthic algae were common.

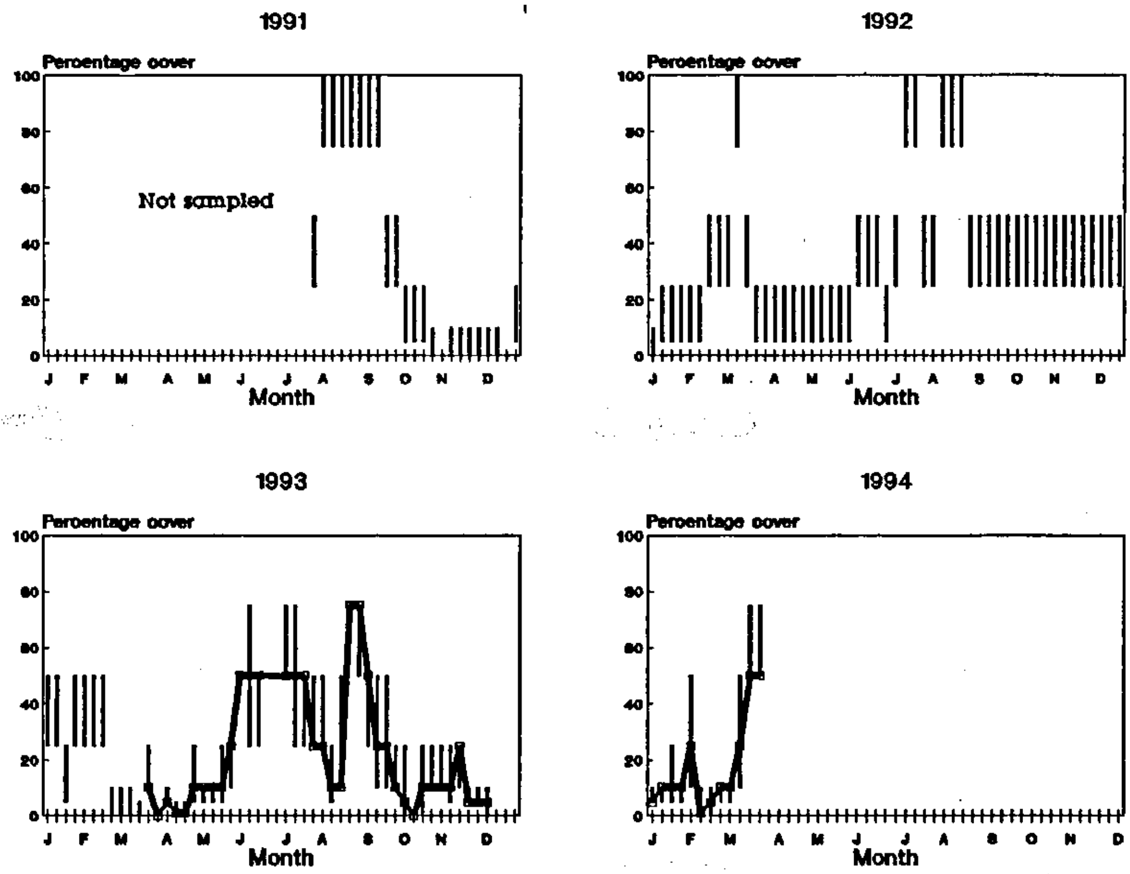


Figure 22. Weekly changes in the abundance of benthic algae at Gifkloof between July 1991 and March 1994. Abundance was initially ranked on a 4-point scale, but in April 1993 abundance ranking was changed to a percentage cover value.

4.5 Water quality

Water quality affects larvicidal efficacy. Of particular importance are the concentration of dissolved salts (which affects larvicidal dispersal) and the pH (which affects larvicidal breakdown). Aspects of the water chemistry were measured at P. K. le Roux Dam between 1981 and 1983 (Hart et al., 1983), at Upington by the Upington Municipality (Vorster pers. comm.) and at various gauging stations by the Department of Water Affairs, Pretoria.

The water was alkaline (pH 7.6-7.8), with a moderate conductivity (15 to 30 mS/m), and a moderate concentration of dissolved salts (140-214 mg/l), dominated by calcium carbonates (73 to 104 mg/l; Table 7). Sodium and chloride levels were low, and nutrient levels, with the exception of nitrate/nitrite, were generally low (Table 7). Both temephos and *B.t.i* should be relatively stable under these conditions. However, in March 1993 a pH exceeding 9 was measured at Westerberg by Dr D. Kurtak, and under these conditions, the hydrolysis of temephos is likely to be rapid (CYANAMID, undated brochure).

Table 7. Chemical characteristics of the Orange River at Marksdrift (MD), Boegoeberg Dam (BB) and Vioolsdrift (VD) measured between January 1980 and October 1993. Data supplied by the Department of Water Affairs, Pretoria.

Determinant		min	med	max	n	Determinant		min	med	max	n
pH	MD	5.3	7.6	8.6	134	NO3+NO2 (mg/l)	MD	0.00	0.48	3.11	134
	BB	6.7	7.9	8.7	118		BB	0.01	0.35	0.93	113
	VO	6.3	7.8	8.7	222		VO	0.00	0.06	3.09	219
Conductivity (mS/m)	MD	8.0	18.0	97.7	372	NH4 (mg/l)	MD	0.00	0.04	0.98	133
	BB	17.4	22.1	54.8	269		BB	0.00	0.05	0.24	113
	VD	-	29.8	77.7	629		VD	0.00	0.04	0.19	216
Total dissolved salts (mg/l)	MD	87	140	191	133	PO4 (mg/l)	MD	0.000	0.016	0.816	133
	BB	123	172	367	113		BB	0.001	0.016	0.046	113
	VD	132	217	414	216		VD	0.000	0.015	0.167	216
CaCO3 (mg/l)	MD	42	73	98	134	Ca (mg/l)	MD	14	19	25	133
	BB	58	85	112	113		BB	15	21	38	113
	VD	65	104	162	219		VD	18	26	43	216
Na (mg/l)	MD	4	7	18	133	Cl (mg/l)	MD	2	6	15	133
	BB	6	11	48	113		BB	3	11	60	111
	VD	7	19	55	216		VD	6	16	60	216

5. BLACKFLY LARVAL AND PUPAL ABUNDANCE

5.1 Method of assessing blackfly larval and pupal abundance

At the beginning of this project, blackfly larval abundance was assessed on artificial gauze substrates. The method worked reasonably well, but was time-consuming, and substrates were often tampered with, stolen, washed away, or left exposed by low water. Furthermore, substrates required two visits per study site, which increased time and costs of sampling. Their use also posed problems regarding species substrate selectivity, and assumptions about colonisation times and equilibrium populations (Resh, 1979; Pegel, 1981; Morin, 1987). In addition, their position in the water, particularly in relation to flow, is critical for uniform colonisation (McCreadie & Colbo, 1991). For these reasons, a rapid and simple method of assessing the abundance of blackfly larvae and pupae on natural substrates was developed.

The method was based on the visual comparison of blackflies on hand-held, natural substrates, to ten diagrammatically prepared abundance classes. Details of the method are published elsewhere (Palmer, 1994), but a brief description is as follows. Two sets of diagrammatic representations for populations of small (2 mm) blackfly larvae and pupae in each of 10 abundance classes were prepared. One set was prepared for use on large, flat surfaces, such as stones and broad leaves (Figs. 23 & 24), and the other was prepared for use on thin, cylindrical surfaces, such as twigs, roots, grass and trailing vegetation (Fig. 25 & 26). The classes ranged, on a semi-log scale, from no individuals (Class 1) to an excess of 500 000 per m^2 for larvae, and 250 000 per m^2 for pupae (Class 10; Table 8).

Table 8. The number of larvae and pupae per 4 x 4 cm (16 cm^2) in each of 10 abundance classes used for estimating the abundance of immature blackflies. Ranges for the classes are given in brackets.

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	1050 (>800)	600 (>400)

The diagrams were of such a size that when the highest nine classes were placed on a single A4 sheet, the sheet could be held in one hand, and conveniently used in the field. The diagrams were photocopied onto plastic sheets which, together with a coloured backing, were taped onto perspex plates[1]. For the sampling procedure, natural substrates in fast-flowing water were lifted by hand, and population densities were visually compared to the diagrammatic abundance classes. Sampling variability was reduced by choosing substrates from areas with similar hydraulic conditions (i.e., random stratified method; Chutter & Noble 1966; Resh 1979). A wax crayon was used to mark scores (expressed as frequencies) onto the perspex plate.

For flat surfaces, estimates were based on the abundance of blackflies in the area of highest density. A 4 x 4 cm quadrat, cut out from a piece of hard plastic, was used to demarcate the area. Separate areas were used for larvae and pupae. For cylindrical surfaces, abundance was estimated by choosing, in each hand-grab sample, a length of vegetation in which the abundance of blackflies was highest and their distribution uniform. Ideally, a length of about 12 cm of vegetation was used (Figs. 25 & 26). Often, however, larvae and pupae were tightly clumped, and a smaller length (down to 1 cm) of vegetation was used. A sample size of 30 was used in all estimates, unless insufficient substrates were available.

The differences between the median abundance class before and after larvicide applications were used to determine the percentage mortality as follows:

$$\text{Difference} = \frac{Y-X}{Y} \cdot 100$$

where Y = the number of individuals of the median class for the 4 x 4 square before application and
X = the number of individuals of the median class for the 4 x 4 square after application (see Table 9).

e.g., Reduction of class 8 to 5

$$\text{Difference} = \frac{202 - 16}{202} \cdot 100$$
$$= 92\ \%$$

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[1] Perspex counting plates are available at cost from the author or from the Onderstepoort Veterinary Institute.

The main advantage of the visual method over conventional counting methods is that large numbers of samples can be collected quickly. The time taken to assess the abundance of larval blackflies on 30 substrates was about 15 minutes, depending upon substrate accessibility. The method can be used effectively with minimal equipment and little training, although first-time users should test their estimates against actual (counted) values until they are confident that their estimates are realistic.

Table 9. Percentage change in the numbers between abundance classes, used to estimate percentage mortality of blackfly larvae as a result of larvicide applications.

		Median class before									
		1	2	3	4	5	6	7	8	9	10
	1	-	>99	>99	>99	>99	>99	>99	>99	>99	>99
	2		-	66	83	94	97	99	99	99	99
	3			-	50	81	92	97	99	99	99
A	4				-	62	83	93	97	99	99
f	5					-	56	82	92	97	98
t	6						-	59	82	93	97
e	7							-	56	82	92
r	8								-	60	80
	9									-	52

A change in the abundance class from a median of 8 before application to a median of 5 after application represents a larval mortality of 92 %.

One disadvantage of the proposed method is that it is limited to loose substrates (stones and trailing vegetation) no deeper than about arms' length. Samples taken from the edge of large and inaccessible rivers, such as the Orange River, may give biased estimates of blackfly abundance. A further disadvantage of the proposed method is that small larvae (first and second instar) may easily be overlooked in the field. The method therefore provides unreliable results when small larvae are abundant. The method should not be used for estimating overall population densities, and should be limited to estimating abundance within a small (4 x 4 cm) area. Despite the limitations, the visual method is practical, simple and quick, and is suited for use in blackfly control programmes, where rapid assessment of larval and pupal abundance is required.

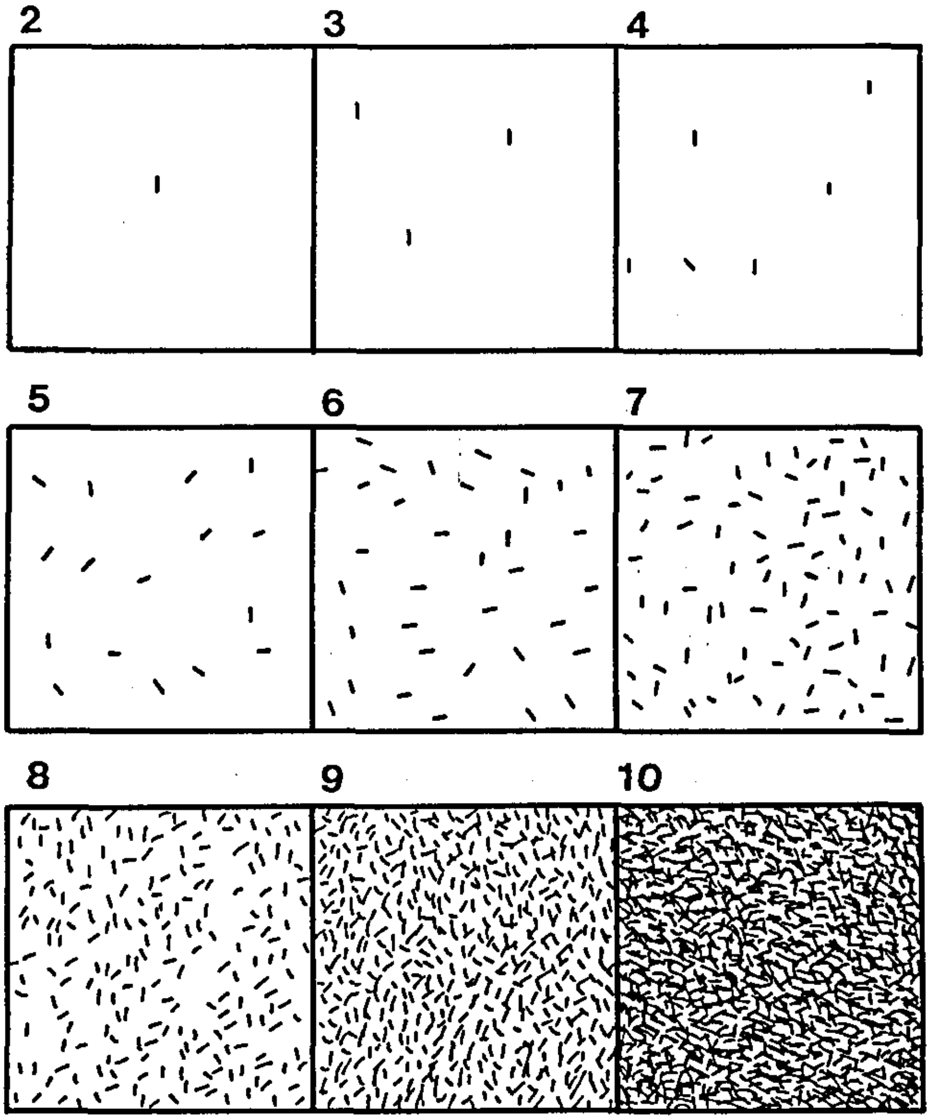

Figure 23. Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of larval blackflies about 2 mm in length found on flat substrates. Classes correspond to numbers shown in Table 8.

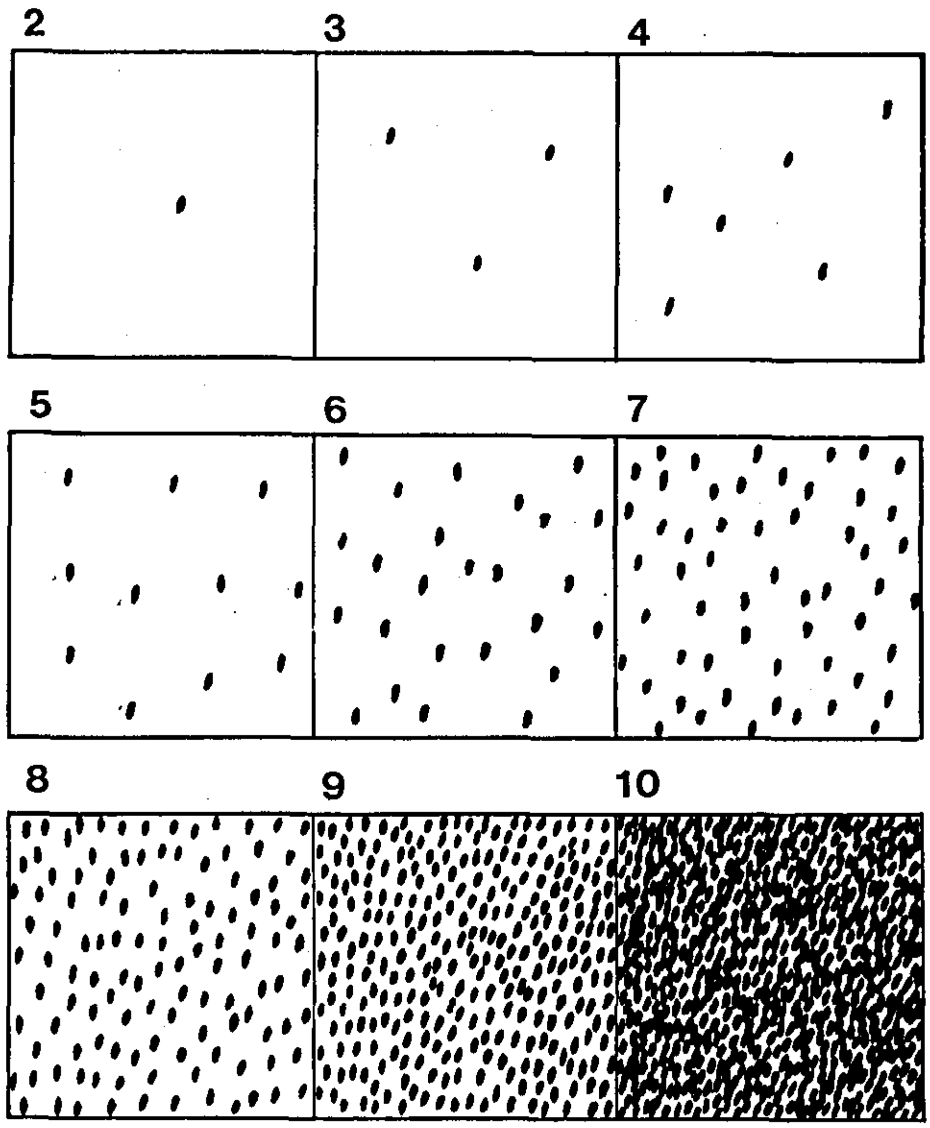

Figure 24. Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of pupal blackflies 2-3 mm in length found on flat substrates. Classes correspond to numbers shown in Table 8.

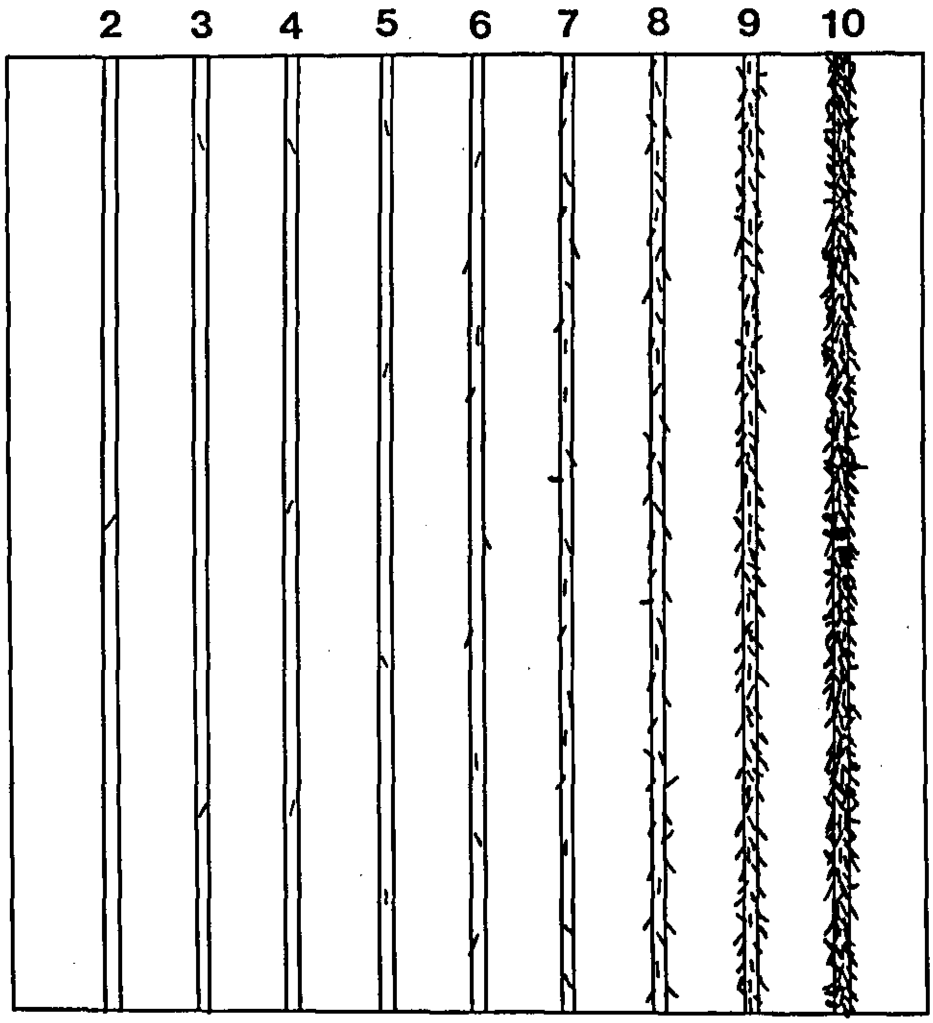

Figure 25. Diagrammatic presentation of semi-logarithmically defined scale for classing population densities of larval blackflies about 2 mm in length found on cylindrical surfaces. Classes correspond to numbers shown in Table 8.

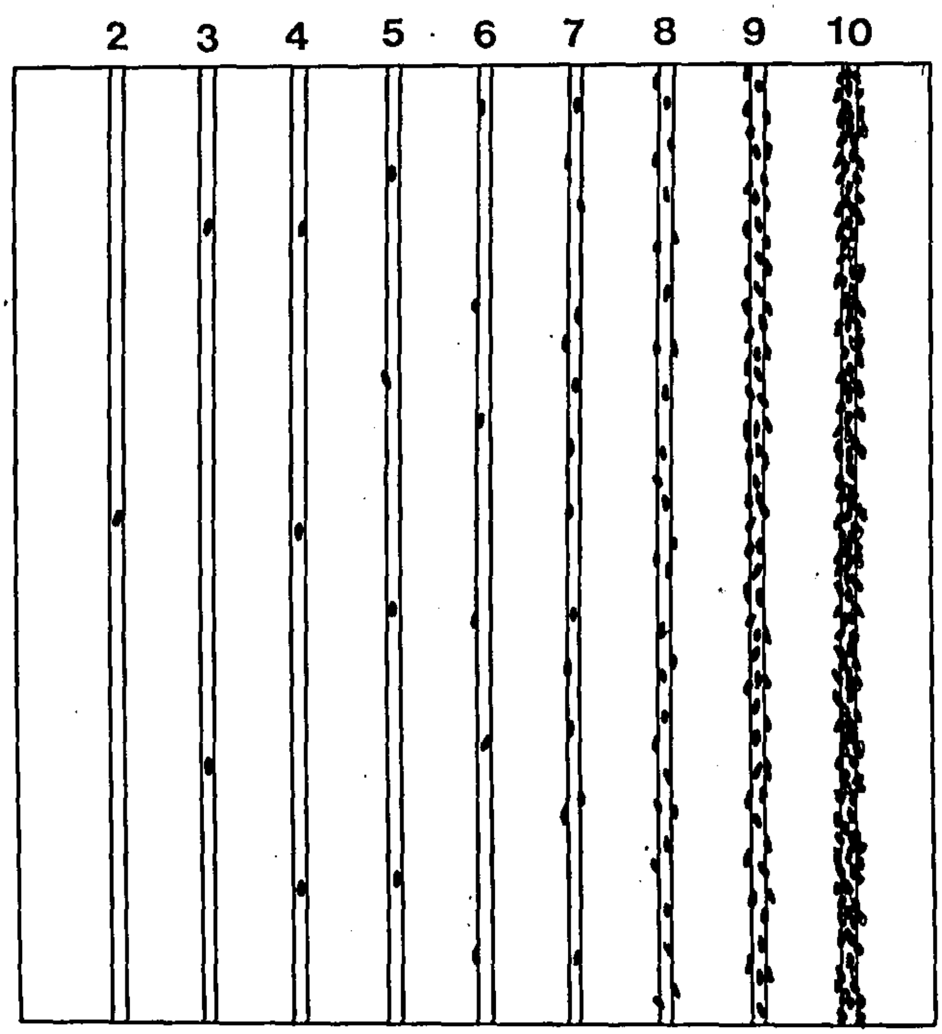

Figure 26. Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of pupal blackflies 2 to 3 mm in length found on cylindrical substrates. Classes correspond to numbers shown in Table 8.

5.2 Results and discussion

The abundance of blackfly larvae and pupae was monitored weekly at Gifkloof. In both 1992 and 1993, larval abundance was lowest in later summer (March and April), and highest in later winter (August and September)(Fig. 27 & 28). The drop in larval abundance in late summer was first thought to be caused by the emergence of adults at a rate greater than the hatching of eggs. However, the very sharp drop in larval numbers in March 1993 coincided with a bloom of the blue-green algae *Microcystis* sp. and *Anabaena* sp., suggested that these algae are toxic to blackfly larvae.

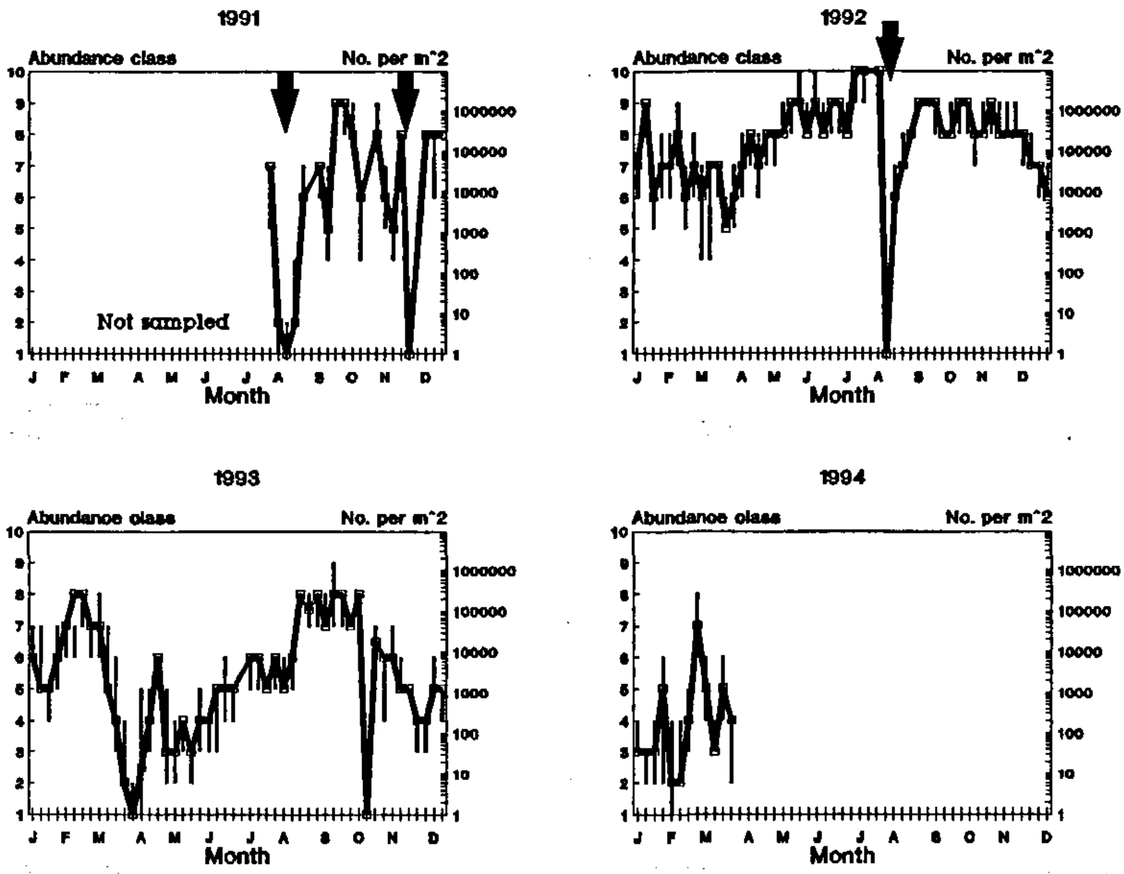

Figure 27. Seasonal changes in the abundance of blackfly larvae at Gifkloof between July 1991 and March 1994. Arrows indicate the timing of larvicides that affected Gifkloof. Data are based on median values. Bars indicate 95 % confidence limits.

The abundance of pupae showed similar trends as the larvae, although there was a puzzling drop in numbers in July 1992 (Fig. 28), at a time when larval numbers were high (Fig. 27). This was the coldest time of the year, and perhaps larval development is so slow at temperatures below 10^{0}C, that larvae do not pupate. However, an extrapolation of the data in Fig. 31 suggests that *S. chutteri* larvae cease to develop at temperatures less than 5^{0}C, which is colder than the Orange River ever gets. Nevertheless, the consistent build-up of larval numbers through winter suggests that larval development at this time of the year is slower than larval recruitment (hatching of eggs).

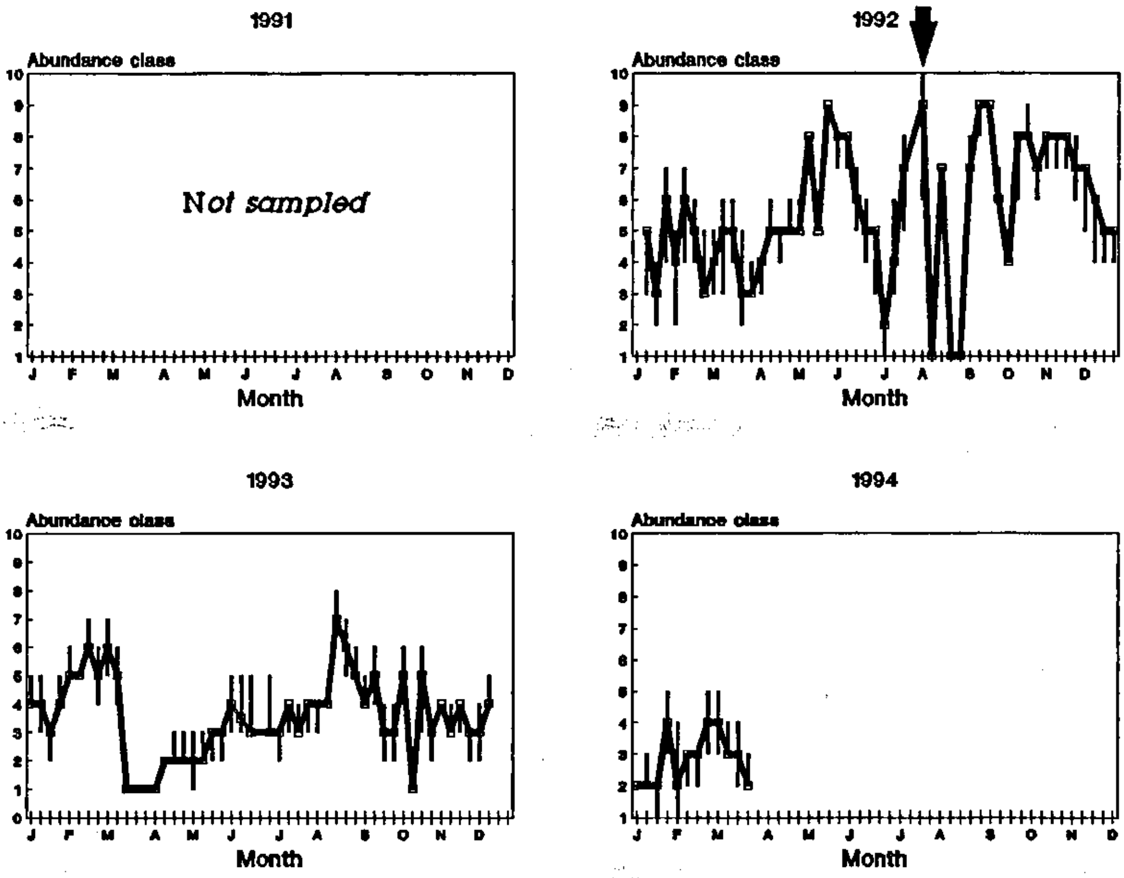

Figure 28. Seasonal changes in the abundance of blackfly pupae at Gifkloof between January 1992 and March 1994. Arrows indicate timing of larvicides that affected Gifkloof. Data are based on median values. Bars indicate the 95 % confidence limits.

With the decreasing water levels in 1993, the weekly sampling site was moved from G2 to G3, and later from G3 to G4 (Appendix C). These changes caused apparent increases in larval numbers, and highlight the problem of obtaining reliable estimates of population abundance. A possible solution to this problem would be to build a sloping embankment which would provide constant hydraulic conditions at all flows.

The drop in larval numbers in October 1993 was because of a sudden rise in flow. This was followed by a spectacular increase in the numbers of *S. damnosum* s.l. found on trailing vegetation at the Upington railway bridge (Fig. 29). The rapid response of this species was unexpected, and suggests that *S. damnosum* s.l. eggs remain in diapause, and develop synchronously after flow increases. In June 1994 flow levels suddenly increased, but blackfly larvae were slow in responding, suggesting that flow

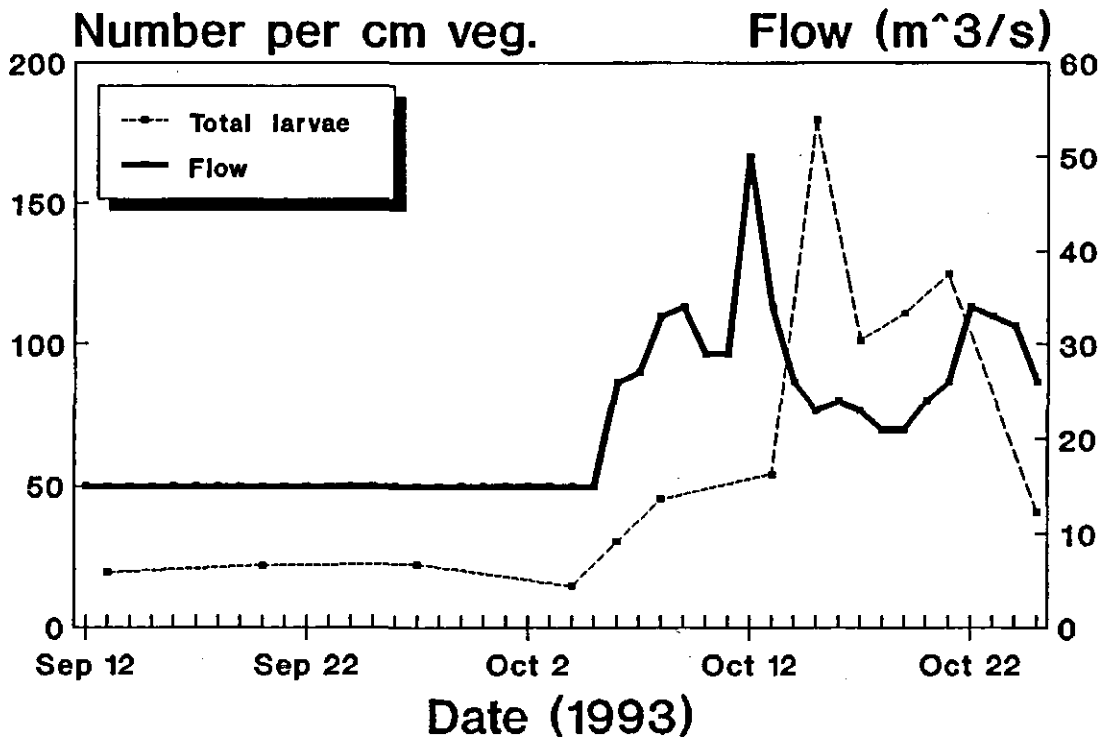

Figure 29. The response of *S. damnosum* s.l. larvae to an increase in flow which occurred on Oct. 5 1993. Large numbers of pupae appeared 10 days after the initial rise in flow. Water temperature was 22-24^{0}C.

alone is not sufficient to trigger hatching. Water temperature in June was cold (<12^{0}C), suggesting that temperature is an important stimulus for hatching.

6. TIMING OF LARVICIDAL CONTROL

Timing of larvicide applications depends on the rate of larval development, which is largely a function of water temperature (Merritt et al., 1982; Ross & Merritt, 1987; de Moor, 1982a; 1994; McCreadie & Colbo, 1991). However, food quality and quantity (Colbo & Porter 1979; 1981; Ross & Merritt, 1987), parasitism, larval density and species may also be important (Crosskey, 1990: 135). Larvicides were applied at various localities in the Orange River in the vicinity of Upington between July 1991 and March 1994. The "development time" was taken as the time required for the first cohort of larvae to pupate following a larvicide application. Sites were visited every 1 to 3 days following application. The recommended treatment interval was defined as the number of days taken for the first cohort to reach 5-6th instar. Degree-day calculations were based on the product of the number of days taken for the first pupae to appear, and the average (maximum-minimum) water temperature recorded weekly over the same period (McCreadie & Colbo, 1991). On one occasion, instar-frequency distributions were determined every 2 to 3 days from head-capsule measurements of 50 randomly selected larvae. Instars of *S. chutteri* were identified using the keys prepared by de Moor (1982a).

The time taken for blackfly larvae to develop to pupae following larvicidal treatments in the Orange River varied from 7 to 37 days (Table 10). Temperature explained most of the variation, although seasonal changes in food quality and quantity were probably also important. Despite the large variances, there were consistent trends which enable generalisations to be made. Firstly, development following multiple-site applications of *B.t.i.* was longer than after single-site applications. This was first noticed following the first large-scale larvicide application in July 1991. The first *S. chutteri* larvae (mostly 3rd instar) were noticed 19 days after application, and the first pupae appeared after 37 days (Fig. 30). First and second instar larvae were presumably present before Day 19, but were overlooked because of their small size. It was suspected that the delayed development was because of residual toxicity of *B.t.i.* applied upstream. Preliminary tests on the persistence of *B.t.i.* toxicity supported the suspicion (Chapter 7.2). The results indicate that single-site applications underestimate the appropriate timing of subsequent large-scale control treatments.

Secondly , the number of degree-days required for *S. chutteri* larvae to develop is relatively constant at about 200 to 300 (Table 10). However, more degree-days are required at colder temperatures (<15^{0}C) than at warmer temperatures. Degree-days

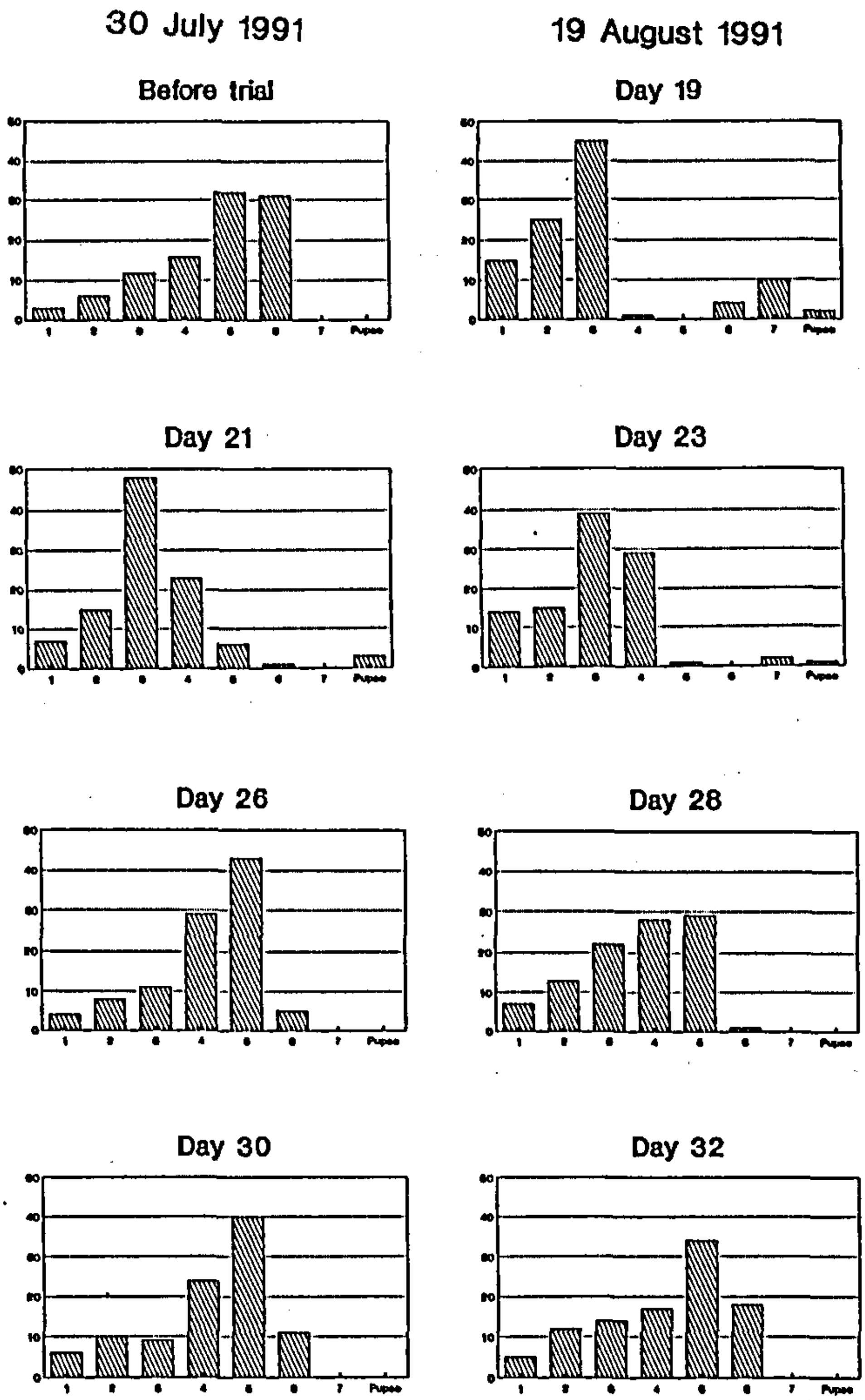

Figure 30. Instar-frequency distributions of *Simulium chutteri* before and after *B.t.i.* application in the Orange River on 30th July 1991. Water temperature during this period was 10-16^{0}C.

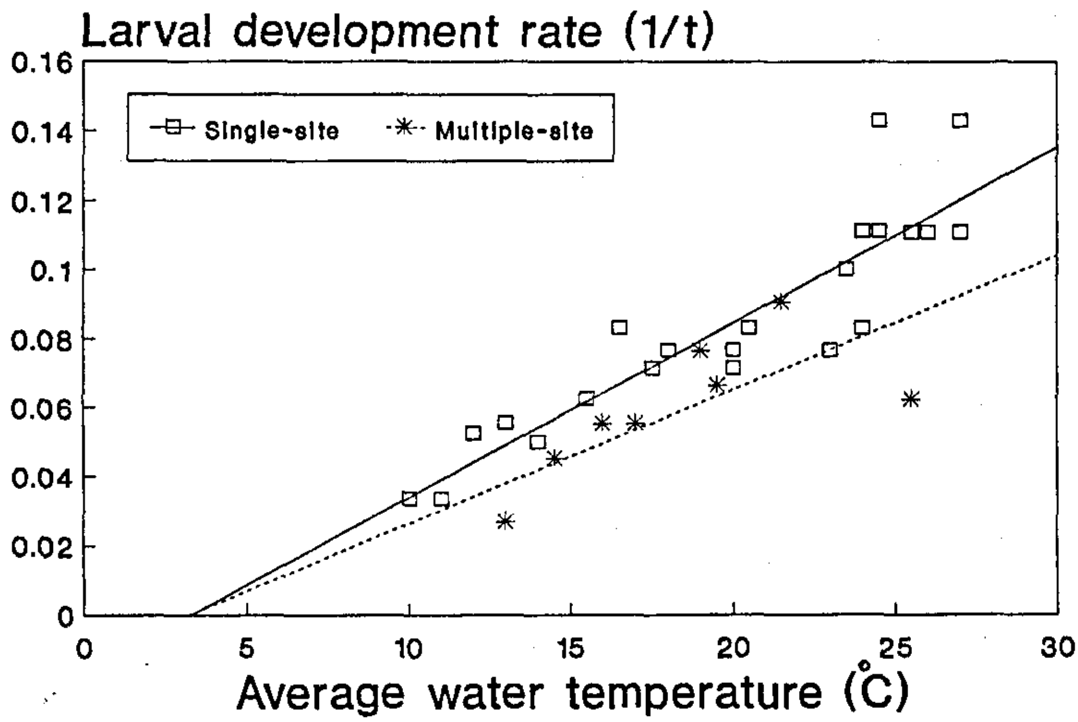

Figure 31. Rate of development of *S. chutteri* larvae following field applications of larvicides in the Orange River, expressed as a function of average water temperature recorded during the period of development.

therefore provides a useful guide for estimating appropriate treatment intervals, but care should be taken at low temperatures. For example, blackfly larval development is usually complete within 6-14 days at high water temperatures (>25°C), but may take up to 70 days at cold temperatures (9-15.5 °C; Smart, 1934, in Crosskey, 1990).

Thirdly, although water temperature provides reasonably accurate information for the correct timing of treatments, the *actual* timing of treatments is often based on practical considerations. For example, although development times of the pest *S. damnosum* s.l. in West Africa are generally 8-12 days in the wet season and 11-14 days in the dry season (Crosskey, 1990: 137), larvicides are applied at 7 day intervals to ensure that no pupae are formed, even if control is delayed by a few days because of logistical problems (Kurtak pers. comm.). Likewise, blackfly control along the Orange River usually started on a Monday because of various practical reasons, and this restricted treatment intervals to a multiple of 7 days.

Although it is tempting to use a longer rather than shorter treatment interval, a single adult female *S. chutteri* probably lays 500 to 1000 eggs in a life-time (unpublished data), and the extra days thus gained do not compensate for the reduced efficacy of control because of emerged (escaped) adults. It is therefore recommended that shorter rather than longer treatment interval is used, even though this may necessitate extra treatment. Recommended intervals for treatment are shown in Table 11.

Table 10. Details of the development times of blackfly larvae following larvicide applications under various conditions in the Orange River, arranged in order of increasing average water temperature recorded during the time of development.

Date	Larvicide	Temp. (^{0}C) min-max.	Av.	Spot	Flow (m^3/s)	TSS (mg/l)	Algae (cells/ml)	Days to pupation	Degree days	Recommended appl. int. (Days)
16.06.92	Teknar^{R} HP-D	8-12	10.0	11	89	52	-	30	300	28
22.05.92	Teknar^{R} HP-D	10-14	12.0	14	90	50	-	19	228	19
30.07.91	Teknar^{R} HP-D	10-16	13.0	12	120	59	-	37	481	32*
03.08.92	Abate^{R} 200EC	9-17	13.0	12	92	41	-	32	416	30*
28.07.93	Teknar^{R} HP-D	10-15	13.0	14	16	8	130	18	237	18
07.07.93	Vectobac^{R} 12AS	12-16	14.0	13	18	14	200	20	270	19
10.08.93	Teknar^{R} HP-D	11-18	14.5	14	15	7	680	22	304	21*
27.04.92	Abate^{R} 200EC	14-17	15.5	17	116	51	-	16	248	14
24.08.92	Teknar^{R} HP-D	12-20	16.0	14	67	29	-	18	288	18*
07.05.93	Teknar^{R} HP-D	15-18	16.5	18	29	23	840	12	198	11
25.08.93	Teknar^{R} HP-D	14-20	17.0	14	10	12	380	18	318	17*
04.09.92	Vectobac^{R} 12AS	15-20	17.5	17	63	24	-	14	245	13
01.09.93	Abate^{R} 200EC	16-20	18.0	18	10	17	380	13	238	12
21.09.92	Teknar^{R} HP-D	15-23	19.0	20	59	26	-	13	247	13*
08.09.93	Vectobac^{R} 12AS	17-22	19.5	17	12	12	690	15	279	14*
14.10.92	Abate^{R} 200EC	18-22	20.0	21	65	44	-	13	260	11
02.04.93	Abate^{R} 200EC	15-25	20.0	25	22	14	5,200	14	309	13
25.09.92	Abate^{R} 200EC	18-23	20.5	19	59	29	-	12	246	12
25.09.93	Teknar^{R} HP-D	20-23	21.5	22	15	13	680	11	222	11*
11.11.92	Vectobac^{R} 12AS	21-25	23.0	23	54	44	-	13	299	11
16.03.93	Abate^{R} 200EC	21-26	23.5	26	29	25	10,000	10	235	10
15.03.94	Abate^{R} 200EC	22-26	24.0	24	80	41	5,080	12	288	11
07.12.92	Abate^{R} 200EC	23-25	24.0	24	63	44	-	9	216	7
25.01.93	Abate^{R} 200EC	23-26	24.5	24	68	34	-	9	220	8
01.12.93	Vectobac^{R} 12AS	22-27	24.5	27	45	-	-	7	196	7
25.11.91	Teknar^{R} HP-D	21-26	25.5	23	295	86	-	16	376	14*
01.02.93	Vectobac^{R} 12AS	24-27	25.5	24	50	31	-	9	230	8
04.02.92	Abate^{R} 200EC	25-27	26.0	25	164	70	-	9	234	7
22.02.94	Abate^{R} 200EC	26-28	27.0	26	104	67	12,060	9	243	8
04.01.93	Abate^{R} 200EC	25-29	27.0	26	56	31	-	7	189	6

"Spot" temperature refers to the water temperature measured at the time of application. Degree-days were based on maximum-minimum temperatures recorded weekly. Recommended treatment intervals are indicated (in days). Stars (*) indicate multiple-site applications.

Table 11. Calender showing the recommended treatment intervals (in days) for the control of *S. chutteri* in the Orange River.

	DAY OF THE MONTH																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Jan	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-
Feb	8	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-
Mar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apr	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-
May	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	16	-	-	-	-	-	-	-	-
Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jul	-	-	-	-	-	-	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18	-	32*	-
Aug	-	-	30*	-	-	-	-	21*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18*	17*	-	-	-	-	-	-
Sep	12	-	-	13	-	-	-	14*	-	-	-	-	-	-	-	-	-	-	-	-	13*	-	-	-	12	11*	-	-	-	-	-
Oct	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nov	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	14*	-	-	-	-	-	-
Dec	7	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stars (*) indicate multiple-site applications. Times of potential outbreaks are printed in bold.

7. LARVICIDAL "CARRY" AND EFFICACY

The distance between successive larvicide application points is an important consideration in the control of pest blackflies. This distance is determined by the downstream "carry" of larvicides. Many factors affect "carry". In general, small rivers have short "carry", mainly because of a low depth:width ratio (Undeen & Colbo, 1980; Undeen et al., 1984) and the increased retention capabilities of the hyporheic zone (Tousignant et al., 1993). Small rivers therefore require more application points than large rivers, and the same river at low flow may require more application points than at high flow (Colbo & O'Brien, 1984). Downstream "carry" is also affected by the physical and chemical conditions in the river. In particular, "carry' is greatly reduced by the presence of pools (Molloy & Jamnback, 1981; Colbo & O'Brien, 1984; Lacey & Undeen, 1986) and aquatic vegetation (Frommer et al., 1981a & b; Undeen et al., 1984; Lacey & Undeen, 1984; 1986) and periphyton (Tousignant et al., 1993).

In 1983 *B.t.i.* (Teknar^R, rated at 1500 AAU) was tested against *S. chutteri* in the Orange River: it was effective for 6 km at a flow of 38 m^3/s (de Moor & Car, 1986). In large rivers (457 m^3/s) *B.t.i.* may "carry" for up to 32 km (Lacey et al., 1982). Temephos, on the other hand, "carries" further than *B.t.i.*, and is effective for up to 40 to 50 km in large rivers (200 m^3/s; Kurtak, Jamnback, Meyer, Ocran & Renaud, 1987). Larvicidal "carry" and efficacy is also affected by the particular formulation used (Guillet & Escaffre, 1979; Lacey & Undeen, 1984; Lacey & Heitzman, 1985), as well as the dosage and duration of application (Frommer et al., 1983; Muirhead-Thomson, 1983; Lacey & Undeen, 1984).

The aim of this chapter was to determine the "carry" and efficacy of *B.t.i.* and temephos under various conditions in the Orange River.

7.1 Methods

Trials were conducted in six zones in the Orange River in the vicinity of Upington (Table 4; Fig. 6). Zones were chosen on account of the access to and availability of sampling sites. Larvicides were applied undiluted directly across the river from bridges, a boat or a canoe. In addition, a Bell^R Jet-Ranger helicopter, fitted with a Simplex^R spray tank with a capacity of 400 l, was used in trials conducted during large-scale control operations. The helicopter applied larvicides in a z-pattern across the river, usually in less than 1 minute. Applications by boat took up to 10 minutes (Table 12). Multiple-site applications were usually conducted during large-scale control operations, but on two occasions, "large-scale" applications were simulated by treating at least two additional sites at 5-6 km intervals upstream of a single-site application point.

Larvicidal efficacy was determined by assessing the abundance of blackfly larvae at 3 to 7 sites downstream of the points of application, directly before and one to three days after each application. "Carry" was defined as the downstream distance that larvicides caused >80 % blackfly larval mortality. Details of "carry" trials conducted in the Orange River are provided in Table 12 and 13.

In addition to "carry" trials, the dispersal properties of each new batch of larvicide used in the Orange River was observed in a glass cylinder. The cylinder was 0.1 x 0.1 x 1.05 m in size, and was filled with river water to a depth of 1 m. A syringe was used to apply 2 ml of larvicide from a height of 0.2 m above the water surface. The time taken for larvicides to disperse between 0.2 and 0.9 m depth was used as an index of dispersal rate. Water temperature and the presence of heavy droplets were noted.

The distance upstream of rapids that larvicides should be applied was determined by observing the movement of dye (Cartosol Red) through rapids. Dye was applied at various distances upstream of rapids.

7.2 "Carry" of *B.t.i.*

A total of 27 "carry" trials were conducted with *B.t.i.*, of which 6 were multiple-site applications (Table 12). "Carry" of *B.t.i.* was usually about 5 km, and up to 20 km at high flow (Figs. 32 & 33).

The first trial, conducted during the first large-scale aerial control operation in July 1991, was highly successful, with >95 % blackfly mortality for 15.5 km (at a flow of 113 m^3/s). This trial was followed by a series of unsuccessful applications from bridges and a boat ("carry" trials No. 2, 3 and 5). The reasons for these failures were bad selection of sampling sites (which were either too near or too far from the point of application) and/or the long duration of application. The small boat we used could carry only half the larvicide required, and the site of application (where the river was a single channel) was 300 m upstream from the only available launching site. This meant that there was a 10-15 minute delay during application. Furthermore, larvicides were applied over the recommended 10 minute interval, which is clearly too long for such a large river.

The results of trial No. 7 (at Gifkloof) were uncertain because of the simultaneous application of temephos, applied 69 km upstream of Gifkloof (at Grootdrink) during large-scale control operations. Interference was suspected because no moribund larvae were present, indicating that temephos (and not *B.t.i.*) was

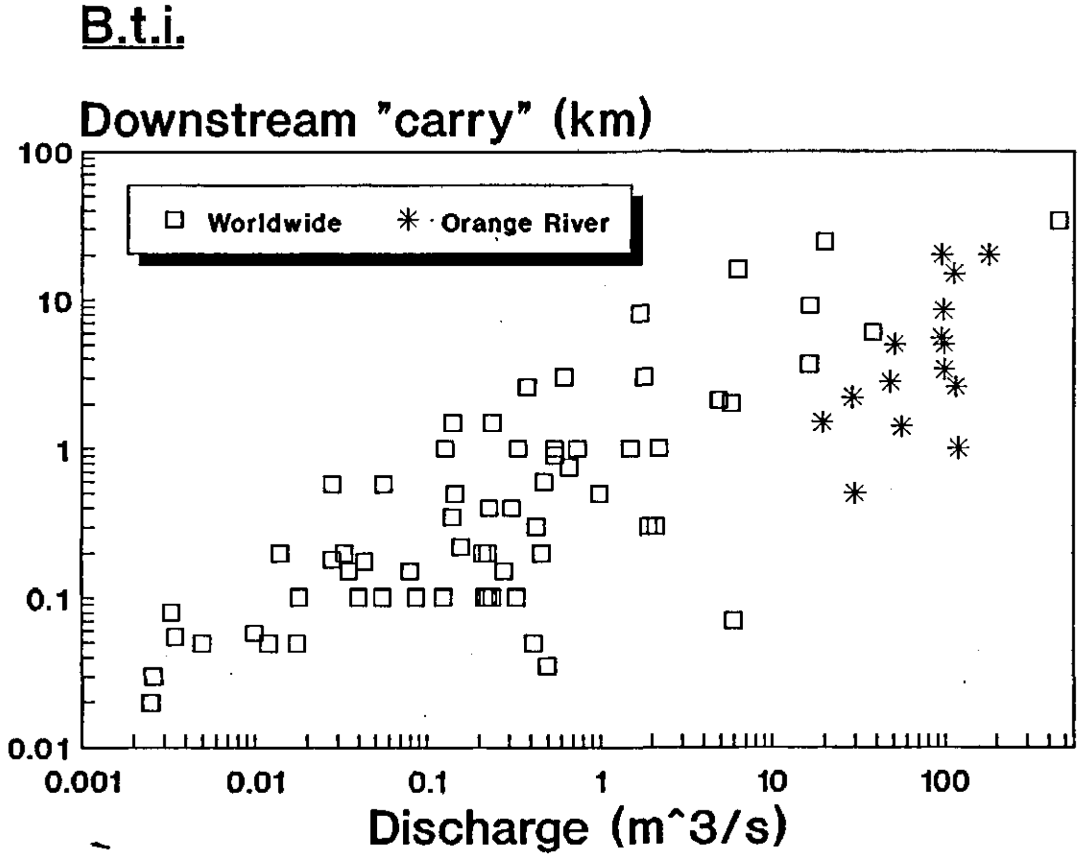

Figure 32. Downstream "carry" of *B.t.i.* as a function of river discharge worldwide.

responsible for their disappearance.

A high-dosage application (2.0 ppm; Trial No. 9) from the Upington Bridge when the flow was high (220 m^3/s), was effective for between 15.8 and 28 km. A subsequent high-dosage trial (No. 15) at a lower flow (96 m^3/s) was effective for between 5 km (left bank) and 12 km (right bank). At very low flow (6 to 10 m^3/s), high dosages (3.0 to 5.5 ppm) were effective for 1.2 to 3.2 km (Trials No's 37, 39-41).

A low-dosage application (0.8 ppm; Trial No 10) at moderate flow (120 m^3/s) was effective for 1 km, with partial control (59 %) at 2.6 km. Subsequent low-dosage applications were effective for 0 to 1.5 km (Trials 13, 25 & 26). Poor "carry" in trials No. 23 and 26 were attributed to pools which formed at low-flow.

In March 1993 the blue-green algae *Microcystis* sp. and *Anabaena* sp. bloomed in the river, and the number of algal cells exceeded

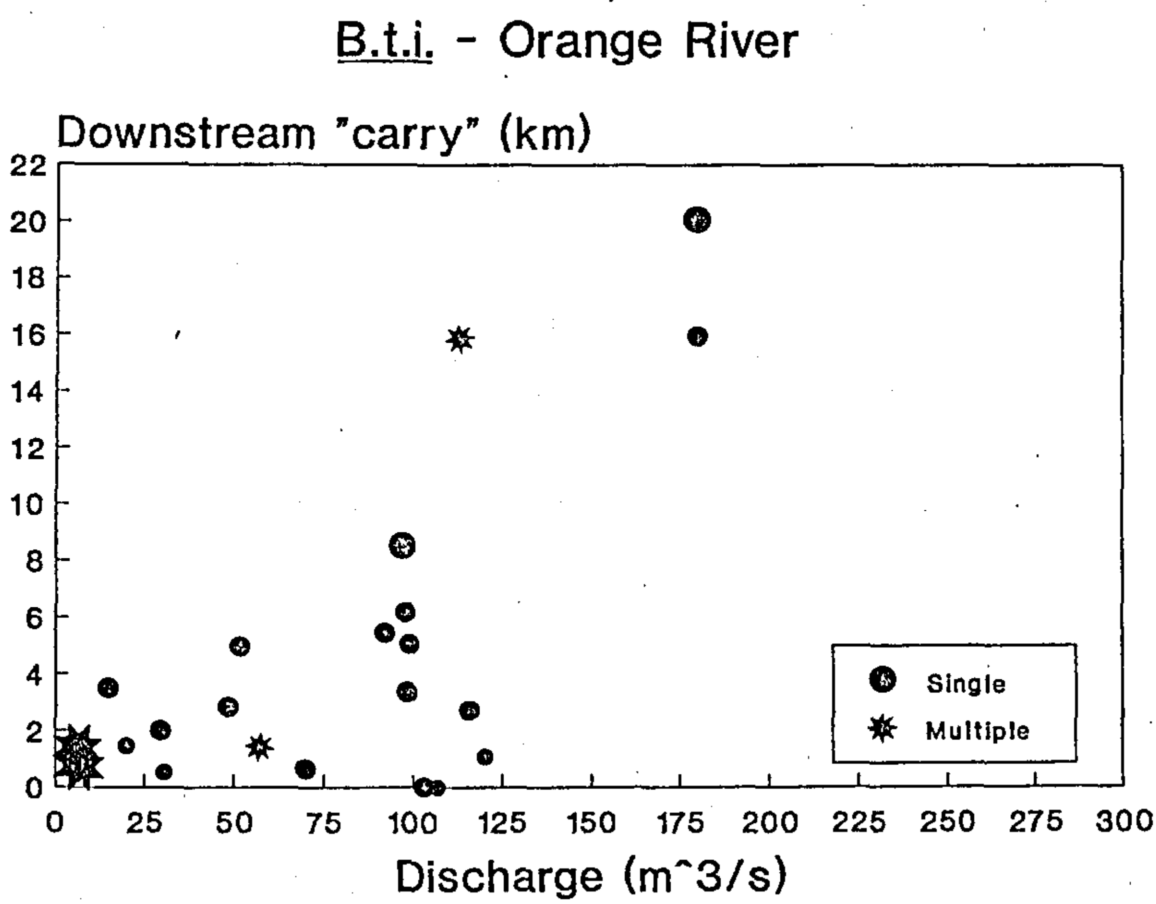

Figure 33. Downstream "carry" of *B.t.i.* as a function of discharge in the Orange River. The size of symbols indicates dosage. (See Table 12 for detailed results.)

15 000 per ml (Fig. 20). A trial conducted under these conditions (No. 34) was unsuccessful, and caused 52-66 % mortality at 2.2 km downstream. Likewise, *B.t.i.* applied from Grootdrink Bridge in February 1994, when the planktonic algal concentration was 12060 cells/ml, did not reach the first sampling site, 3.5 km downstream.

7.3 "Carry" of temephos

A total of 23 "carry" trials were conducted with temephos, of which 5 were multiple-site applications (Table 13; Figs. 34 & 35). Temephos "carry" was roughly three times that of *B.t.i.*, and carried for up to 50 to 70 km at flows between 92 and 298 m^3/s (Figs. 34 & 35).

These trials were followed by two trials (Nos. 4 & 6), conducted from the Upington and Keimoes Bridges in November 1991, when flow was high (298 and 293 m^3/s respectively). "Carry" from the

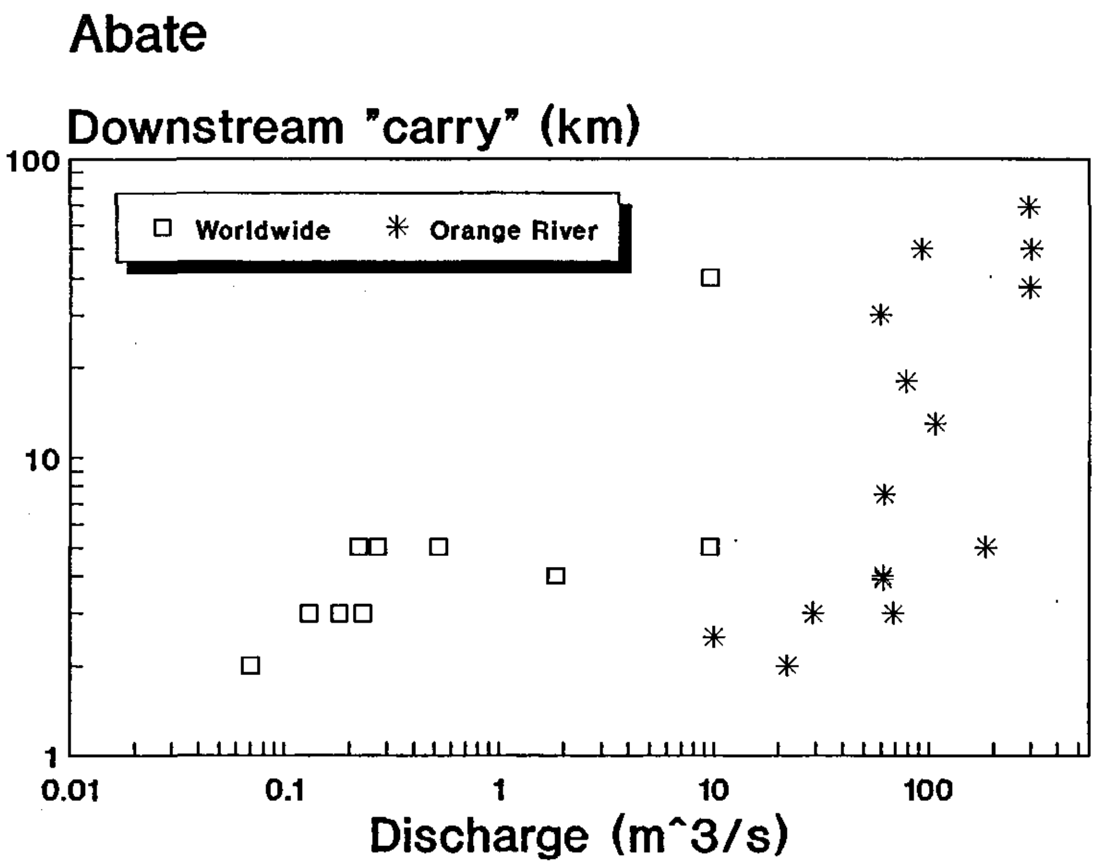

Figure 34. Downstream "carry" of temephos as a function of river discharge worldwide.

Upington Bridge was between 45.1 and 56.2 km. Larval mortality at the Kakamas Bridge, 37 km downstream of the Keimoes Bridge, was 100 %, indicating "carry" of at least 37 km (Table 13).

Temephos was next applied to the Orange River at, and upstream of, Groodrink, during large-scale control operations in November 1991. These applications were not intended as a "carry" trial, but they interfered with a *B.t.i.* trial 69 km downstream, at Gifkloof Weir (Table 12). Flow at the time was high (290 m^3/s).

The following temephos trial (No 8) was conducted from the Upington Bridge in January 1992, when flow was still high (184 m^3/s). Recommended dosages were halved to 0.05 ppm, and "carry" was about 5 km (Table 13). Flows in the river began to drop. In May 1992 a trial from the Upington Bridge was conducted at a flow of 107 m^3/s (Trial No. 12). Mortality at the first sampling site, 0.8 km downstream, was low (56 %), but reached

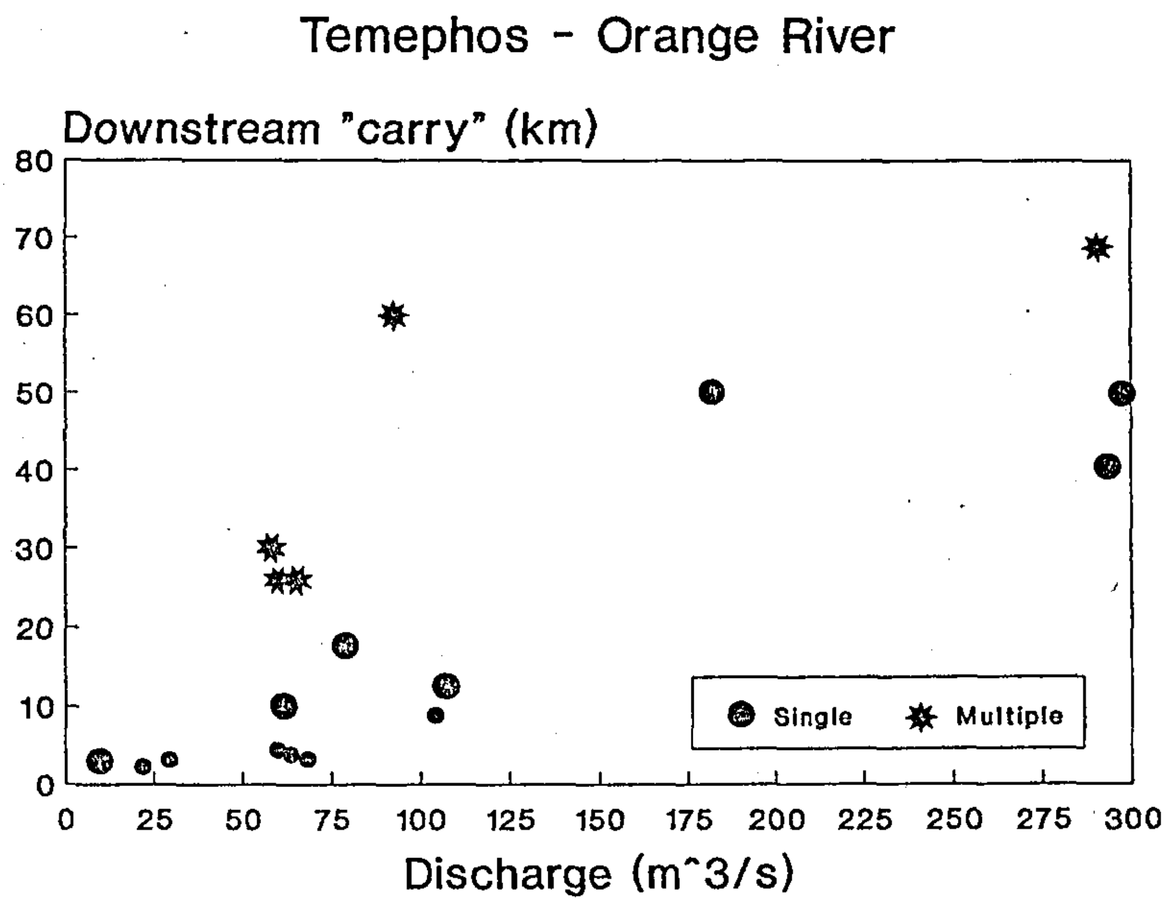

Figure 35. Downstream "carry" of temephos as a function of discharge in the Orange River. The size of the symbols indicate dosage.

>99 % at 4.3 km downstream. Effective "carry" was about 13 km (Table 13).

The following temephos trial (No. 20) was conducted during large-scale control operations in mid-winter, when spot water temperatures were 12 ^{0}C (Table 13). "Carry" was at least 50 km at a flow of 95 m^3/s. A single-site application was then performed from the Upington Bridge in September 1992 (Trial No. 21). Flow at the time was 78 m^3/s, and larval mortality 15.8 km downstream was 92 %. Estimated "carry" was 18 km. The next temephos trial was conducted in the same place and under similar conditions, except that larvicides were applied at two additional sites at 5 km intervals upstream of the Upington Bridge (Pokkies Eiland and Uitkoms), to simulate an operational (multiple-site) application. At 48 hrs, larval mortality approached 93 % 22 km downstream, and at 72 hrs mortality exceeded 99 %. At 28 km downstream, larval mortality was 82 %, and the "carry" was estimated as 30 km.

A series of trials was then undertaken to determine the lowest dosage under which temephos was effective (Trials No's. 27-30 & 32). Temephos applied at 0.005 and 0.01 ppm had no detectable effect on larval populations, but worked well at dosages >0.04 ppm.

A trial at Westerberg in March 1993 was not effective (<50 % mortality) within the first 0.7 km. Thereafter, larval mortality exceeded 99 % for at least 7.5 km.

Three trials conducted at a low flow (14 to 26 m^3/s) at Sonesta gave similar "carries" of 2 to 3 km.

7.4 Dispersion

Both Teknar® HP-D and Vectobac® 12AS dispersed rapidly, irrespective of water temperature (tested between 10 and 30^{0}C), and fell between 20 and 40 cm/minute. Vectobac® 12AS, however, was characterised by numerous small droplets which sank rapidly from suspension (at 100 cm/minute). These droplets would presumably be unavailable for ingestion by blackfly larvae.

The formulation of Abate® 200EC, on the other hand, was extremely buoyant, and remained in a thin layer on the surface for 18 hrs, dispersing throughout the water column after 24 hrs only. Experiments with temephos in the Orange River showed that full mixing occurred after about 800 m downstream of the point of application (at a flow of 60 m^3/s).

7.5 Dye trials

Observations on the movement of dye in the Orange River indicated that each set of rapids are unique, and it is impossible to prescribe recommended distances upstream of rapids at which larvicides should be applied. Furthermore, strong winds were found to affect application. In one experiment a strong crosswind blew the dye, which had been applied as a single stripe across the river, all to one bank. In another experiment, winds blew the dye upstream of the point of application! These observations indicate that winds are likely to greatly affect the application of larvicides, such as temephos, which remain on the surface for extended periods.

7.6 Discussion

Results of "carry" trials were highly variable because trials were done at different times and places and under different conditions (flows, temperatures, dosage rates, treatment durations, methods of application etc). The "carry" results from the Orange River are lower than those reported in the literature (Fig. 32). The most likely reason for this are the long stretches of deep pools between the rapids in the Orange River, and the abundance of *Phragmites* spp. reeds.

Another significant finding of this project was that the "carry" of single-site applications was less than that of multiple-site applications, indicating accumulative effect of multiple-site applications. This means that the results of single-site applications underestimate the effects of operational treatments. Therefore, dosages during operational treatments could be reduced. Despite the variable results, a rough idea of likely "carry" distances is provided in Table 14.

Experiments on mixing in streams indicate that buoyant substances (such as the present formulation of Abate® 200EC) greatly enhance transverse mixing. This means that less effort is required to accurately apply temephos than *B.t.i.*. However, Molloy et al. (1984) found that the settling rate of Teknar® was very slow (<5 mm/h). It is therefore important that the settling properties each new batch of larvicide are tested in the river in which they are to be used.

Table 12. Details of larvicide "carry" trials conducted in the Orange River between June 1991 and March 1994 using *B.t.i.*, arranged in order of decreasing river discharge.

	Trial No.	Date	Site	Method	Duration (min)	Vol. (l)	Conc. (ppm)	Flow (m^3/s)	Temp (^{0}C)	TSS (mg/l)	"Carry" (km)	Comments
(T)	5.	22.11.91	Keimoes	Bridges	2	220	1.3	288	24	86	?	Sampling sites too far apart
(T)	7.	27.11.91	G1	Helicopter	1	220	1.3	290	24	85	?	Interference by temephos
(V)	42.	18.02.94	Upington	Bridge	2	130	1.2	181	28	50	16	
(T)	9.	04.02.92	Upington	Bridge	10	220	2.0	180	28	82	20	"Carry" between 15.8 and 28 km
(T)	10.	23.04.92	Upington	Bridge	2	56	0.8	120	22	59	1	Kill 59 % at 2.6 km; Too dilute
(T)	11.	27.04.92	Upington	Bridge	2	84	1.2	116	18	51	2.6	Kill 30 % at 4.6 km
(T)	1.	31.07.91	G1	Helicopter	1	80	1.2	113	13	59	15.9 *	-
(T)	13.	12.06.92	Upington	Bridge	2	51	0.8	106	11	56	0	Mortality 59 % at 0.8 km
(V)	44.	22.02.94	Grootdrink	Bridge	1	73	1.2	104	26	67	0	Poor "carry" : high algal count
(V)	17.	04.09.92	G3	Boat	4	71	1.2	99	17	24	3.4	"Carry" between 2.1 and 4.7 km
(V)	18.	04.09.92	Upington	Bridge	2	71	1.2	99	17	24	5	-
(T)	14.	15.06.92	Upington	Bridge	2	71	1.2	98	11	52	6.2	Mortality about 5 % at 11.8 km
(T)	2.	28.09.91	G1	Boat	10+10	80	1.4	97	20	62	0	Duration too long
(T)	3.	"	Kanoneiland	Boat/Bridges?	15	"	"	"	"	62	?	As above + poor site selection
(T)	15.	17.06.92	Upington	Bridge	2	115	2.0	96	12	51	8.5	"Carry" right=12 km, left=5 km
(V)	16.	03.08.92	Upington	Helicopter	1	67	1.2	92	12	41	5.5	-
(T)		06.11.90	Sanddraai	Boat	15	51	1.2	70	21	-	0.5	Poor "carry" due to pools
(V)	23.	14.10.92	Upington	Bridge/Boat	5	36	1.1	56	21	31	1.4 *	Poor "carry" due to pools
(V)	22.	13.10.92	Neus	Bridge	4	38	1.2	51	21	23	5	Mortality 60 % at 11.2 km
(V)	31.	01.02.93	Jooste-Eiland	Canoe	4	35	1.2	48	24	31	2.8	-
(V)	26.	10.11.92	Neus(Left)	Bridge	10	14.5	0.8	30	23	24	0.5	Poor "carry" due to pools
(T)	34.	16.03.93	G3	Canoe	4	21	1.2	29	26	26	<2.2	Poor "carry" .. high algal count
(V)	25.	10.11.92	Neus(Right)	Bridge	6	9.5	0.8	20	23	24	1.5	-
(T)	37.	10.08.93	Kanoneiland	Helicopter	1	20	2.2	15	14	18	>3.2 *	Took at least 3 days
(T)	39.	25.08.93	Korra-Eiland	Helicopter	1	..	...	10	18	10	<7.5 *	-
(V)	40.	08.09.93	Kanoneiland(R)	Helicopter	1	11	3.0	6	17	12	1.6 *	-
(V)	41.	"	Kanoneiland(L)	"	"	"	"	"	"	"	1.2 *	Did not exceed 1.5 km

V=Vectobac^R 12AS. T=Teknar^{R}HP-D. "Vol" refers the the volume of larvicide applied. Stars (*) indicate multiple-site applications.

Table 13. Details of larvicide "carry" trials conducted in the Orange River between June 1991 and March 1994 using temephos (Abate[R] 200EC), arranged in order of decreasing river discharge.

Trial No.	Date	Site	Method	Duration (min)	Vol. (l)	Conc. (ppm)	Flow (m^3/s)	Temp (^0C)	TSS (mg/l)	"Carry" (km)	Comments
4.	19.11.91	Upington	Bridge	2	80	0.09	298	24	77	50	"Carry" between 45.1 and 56.2 km
6.	26.11.91	Keimoes	Bridges	?4+2+1	100	0.11	293	24	85	37+	"Carry" at least 37 km
7.	27.11.91	Grootdrink	Helicopter	1	87	0.10	290	24	85	?69 *	Interference with *B.t.i.* trial
8.	29.01.92	Upington	Bridge	10	28	0.05	184	29	72	5	Mortality about 20 % at 15.8 km
43.	18.02.94	Kakamas	Bridge	1	54	0.1	181	28	99	50	
	31.06.90	Sanddraai	Boat	9	42	0.10	140	11	-	0.5	Poor "carry" due to pools
12.	22.05.92	Upington	Bridge	3	32	0.10	107	16	60	13	Mortality 56 % at 0.8 km
45.	22.02.94	Upington	Bridge	1	16	0.05	104	26	67	9	
20.	03.08.92	Grootdrink	Helicopter	1	28	0.10	92	12	41	?50+ *	-
21.	25.09.92	Upington	Bridge	4	25	0.10	78	18	30	18	Mortality 59 % at 22 km
	16.10.90	Sanddraai	Boat	14	24	0.10	70	19	-	0	Poor selection of sampling site
30.	25.01.93	G3	Canoe	10	10.2	0.05	68	26	34	3	-
33.	04.03.93	Westerberg 1	Boat	4	18.6	0.10	62	27	16	7.5+	Mortality 100 % at 7.5 km.
32.	16.02.93	Westerberg 1	Canoe	1	7.7	0.04	61	24	42	3.9	-
29.	25.11.92	G3	Canoe	10	9.2	0.05	61	22	46	4	-
46.	03.03.94	Swartkop	Helicopter	1	21	0.01	60	26	54	26 *	
47.	03.03.94	Augrabies	Helicopter	1	21	0.01	60	26	54	26 *	
24.	20.10.92	Upington	Canoe	3	18.0	0.10	59	21	44	30 *	-
35.	16.03.93	Jooste-Eiland	Canoe	2	4.3	0.05	29	26	26	3	-
27.	21.11.92	Neus(Left)	Bridge	6	0.9	0.01	29	22	34	0	Too dilute
36.	02.04.93	Jooste-Eiland	Canoe	2	3.3	0.05	22	25	14	2	-
28.	21.11.92	Neus(Right)	Bridge	6	0.3	0.005	18	22	34	0	Too dilute
38.	01.09.93	Jooste-Eiland	Canoe	5	3.0	0.10	10	18	17	2.5	Did not carry to 3.8 km

Stars (*) indicate multiple-site applications.

Table 14. Approximate downstream "carry" (in km) of *B.t.i.* and temephos in the Orange River.

Discharge (m^3/s)	*B.t.i.* Single	*B.t.i.* Multiple	Temephos Single	Temephos Multiple
25	2	3	3	7
50	3	4	5	15
75	4	7	10	30
100	6	9	15	40
125	8	12	20	50
150	10	16	25	60
175	15	18	30	65
200	18	20	35	70
225	-	-	40	75
250	-	-	45	80
275	-	-	50	85
300	-	-	55	90

8. EFFECTS OF LARVICIDES ON NON-TARGET ORGANISMS

The aim of this chapter is to address the concern expressed over the safety of *B.t.i.* and temephos used in blackfly control in the Orange River. Detailed results of the short-term (before and after) affects of this study are published in Palmer (1993). The effects of repeated dosages are being studied. Material and data collected during this study will be housed in the Albany Museum, Grahamstown.

This chapter summarises the main points, and focuses on the impacts of larvicides on "non-target" benthic macroinvertebrates living in the stones-in-current biotope (rapids and riffles). This was the obvious starting point for such a study because it was the biotope being treated for blackfly control. Furthermore, the invertebrate fauna living in stones-in-current is diverse and is well-researched compared to the fauna in other riverine biotopes. In addition, stones are discrete and therefore easy to quantify, and rapids and riffles are usually shallow and therefore easy to sample. Benthic invertebrates are ideal tools for examining the short-term environmental impact of larvicides because they respond quickly to pollution, provide a time-integrated index of water quality conditions, and are not subject to diel changes associated with drifting invertebrates.

8.1 Methods

The short-term effects of larvicides were assessed at the recommended dosages, to indicate effects under "operational-" conditions, as well as at high dosages, to indicate the "worst-possible" scenario, in the event of an accident or miscalculation. The effects of larvicide application under the "worst-possible" scenario were determined in a small side-stream on the northern bank of Kanoneiland (28°38'08"S; 21°05'21"E) and in a concrete-lined canal at Straussburg (28°25'56"S; 21°21'49"E). The canal was built to return excess [river] water from a large irrigation canal back to the river. Piles of stones were placed at roughly 10 m intervals across the canal to simulate the stones-in-current biotope. The stones were left for 3 weeks to allow natural colonisation before experiments started. Larvicides were applied to both streams using a garden watering-can. The Straussburg canal was fitted with a Parshall gauging flume for accurate flow determination. Flow in the side-stream at Kanoneiland was estimated three times by measuring the depth at 50 cm intervals, and timing a partly submerged plastic bottle to travel 1.2 m downstream at each depth interval (Gordon et al., 1992).

The effects of larvicide application under "operational" conditions were determined in the above-mentioned streams as well as in the river at Sonesta, situated 4.6 km downstream of the Upington road bridge, and at Gifkloof 2 and 3. Larvicides were applied by watering-can or from a canoe. In addition, a Bell® Jet Ranger Helicopter, fitted with a Simplex® spray tank, was used for applications during large-scale control operations. Field trials are inherently inaccurate (Muirhead-Thomson, 1981), and so laboratory trials on selected species were also conducted to corroborate field results.

In the first trial, macroinvertebrates on each of 20 stones were collected two days before and five days after application, preserved in 80 % ethanol and identified and counted in the laboratory. To determine invertebrate population densities, the surface area of each stone was measured by wrapping each stone in a transparent plastic film (Gladwrap®), weighing the Gladwrap® on a microbalance accurate to 0.0001 g, and then comparing this mass to the mass of Gladwrap® of known area. The method was time-consuming, and was replaced, in subsequent trials, by ranking the abundance of invertebrate taxa, irrespective of the size of the stone, on a 4-point abundance scale (0=none; 1=present; 2=common; 3=abundant). Taxa which were "common" or "abundant" were noted for each stone while in the field. Taxa were then removed with fine-forceps, preserved in 80 % ethanol, and identified in the laboratory. Taxa which were not identified as "common" or "abundant" in the field, scored 1. The method was subjective and depended on experience. For example, ten leeches on a stone would score "3", whereas ten blackfly larvae on a stone would score "1". For this reason, "before" and "after" samples of each trial were always done by the same person. Studies have shown that survival of animals entering drift after treatment is low (Wallace et al., 1973; de Joux, 1977), and so the method assumed that the absence of taxa in "after" samples indicated a *direct* effect of the larvicide.

8.2 B.t.i.

The results of this study show that *B.t.i.* has minimal direct effects on "non-target" fauna, even at very high dosages. In all trials, blackfly larvae were the most susceptible macroinvertebrates. The filter-feeding chironomid *Rheotanytarsus fuscus* was affected in all trials in which it was common or abundant. Repeated applications of *B.t.i.* affected the midge *Xenochironomus* sp. "B". No other taxa were directly affected by *B.t.i.*.

Numerous studies worldwide agree that *B.t.i.* is safe to a wide variety of organisms, including flatworms, molluscs, crustacea, amphibians and fish (Colbo & Undeen, 1980; Ali et al., 1981; Gaugler & Finney, 1982; Undeen & Lacey 1982; Molloy, 1982; 1992; Car & de Moor, 1984; de Moor & Car, 1986; Merritt et al., 1989;

Lacey & Mulla, 1990; Welton & Ladle, 1993; Charbonneau et al., 1994). The only taxa susceptible to *B.t.i.* (at dosages in excess of those normally used for blackfly control) belong to the fly (Diptera) suborder Nematocera, and include the larvae of blackflies (Simuliidae), mosquitoes (Culicidae), mountain midges (Blephariceridae), crane flies (Tipulidae) gall midges (Cecidomyiidae), biting midges (Ceratopogonidae), dixid midges (Dixidae) and certain chironomids (Ali, 1981; Ali et al., 1981; Pistrang & Burger, 1984; Back et al., 1985; Merritt et al., 1989; Car & de Moor, 1984; de Moor & Car, 1986; Wipfli & Merritt, 1994). Certain fish species are also affected by *B.t.i.* at high dosages (Merritt et al., 1989), but this has been attributed to ingredients, such as xylene, used in some formulations, rather than the bacterial toxin itself (Fortin et al., 1986; Snarski, 1990). A trout stream in the Adirondack Mountains, New York, was treated repeatedly with *B.t.i.* for two years, and no changes in the invertebrate community structure (apart from the absence of blackflies) were found (Molloy, unpubulished data). De Joux et al., (1985) found that the application of *B.t.i.* to virgin streams produces an increase in drift 4-5 times its normal value, which is very low compared to values after treatment with temephos (usually 50 times). They conclude that there is no evidence of adverse effects of *B.t.i.* application in the medium-term. Recent studies have shown that blackfly predators (Plecoptera and Trichoptera) were not affected by feeding on *B.t.i.*-killed blackfly larvae (Wipfli & Merritt, 1994).

Despite the undeniable safety of *B.t.i.*, it is worth noting that *S. chutteri* is the only target species out of the seven blackfly species which occur in the middle Orange River. Although the remaining 6 do feed on poultry and livestock, their numbers do not attain pest proportions. Of particular concern is the status of *S. gariepense*, which is endemic to large, turbid rivers in southern Africa. Dr F. C. de Moor, of the Albany Museum, Grahamstown, has suggested that *S. gariepense* be regarded as a threatened species, a veiw which is supported by the data collected during this study.

Furthermore, the application of *B.t.i.* may have indirect, secondary effects, not detected in a short-term (before-after) study. A recent study in North America has found that the application of *B.t.i.* indirectly affects tertiary consumers (Merritt et al., 1991). Blackfly predators may simply starve to death if their food source is depleted by the application of *B.t.i.*. However, the important predators of blackfly larvae in the Orange River (Hydropsychidae and Hirudinea) are generalists feeders (de Moor, 1992), and are therefore likely to be resilient to the selective removal of blackflies following larvicide application.

Under field conditions, most studies agree that *B.t.i.* is no longer toxic to blackflies 3 to 5 days after application.

However, several studies have found that *B.t.i.* toxins are stable in water for at least 3 days (Mulligan et al., 1980; Ignoffo et al., 1981a; 1981b; Guillet et al., 1982b). According to a pamphlet produced by Abbott Laboratories, Vectobac[R] "..... will maintain useful activity for a period of one week when mixed with normal tap water". Preliminary tests with Orange River water kept in the laboratory (i.e., out of direct sunlight) showed that Vectobac[R] 12AS remains toxic to blackflies for at least 14 days. Long-term studies of the persistence of *B.t.i.* found that it remains toxic for at least 60 days (Lacey & Undeen, 1986; Ohana et al., 1987). In one experiment, the toxicity of *B.t.i.* increased after 69 days when kept cool (Du Pont & Boisvert, 1986). The authors attributed this to the presence of viable spores which grew and released new toxins. This means that *B.t.i.* products are capable of growth, despite the inclusion of acid in the formulation, which is intended to prevent fermentation. Although *B.t.i.* is a naturally occurring bacteria, its release into the environment on a large scale (about 17 000 litres per year for the Orange River) could change the natural structure of the bacterial community by dominating or replacing natural populations (Power et al., 1988). A danger of this happening would be the continuous exposure of blackfly larvae to *B.t.i.* toxins, which could lead to rapid development of resistance (Holmes, 1993). However, there are no such reports of this ever happening, and the specific conditions under which *B.t.i.* grows makes it unlikely that live *B.t.i.* would out-compete naturally occurring bacteria.

The reason that *B.t.i.* rapidly looses its toxicity to blackflies in the field is due to its rapid incorporation into the hyporheos and absorption to soil particles (Tousignant et al., 1993). In one experiment, 99.8 % of the *B.t.i.* applied to an artificial pond was found in the mud within 45 minutes (Ohana et al., 1987). In another experiment, sediments exposed to *B.t.i.* were slightly toxic to mosquito larvae, whereas epilithic algae was highly toxic (Tousignant et al., 1993). It is likely that most of the *B.t.i.* applied to the Orange River lands up in the sediments and epilithic algae, although it is difficult to detect because of the background levels of bacteria. The toxins are presumably eventually broken down by sunlight and bacteria into their constituent amino acids.

8.3 Temephos

When temephos was applied to the Orange River at the dosage recommended for the "wet season" (0.05 ppm), good control of blackflies was achieved with minimal direct impact on "non-target" fauna. The most sensitive fauna were all species of blackfly larvae, the mayfly *Baetis glaucus*, and the midges *Rheotanytarsus fuscus* and *Cardiocladius ?africanus* (Table 15). Studies elsewhere have shown that the recommended dosage of temephos (0.05 ppm) is safe for fish, crayfish and stonefly nymphs (Swabey et al., 1967; Tsai, 1978; Lee & Scott, 1989; Mohsen & Mulla, 1981). However, these studies did not address the effects of sublethal dosages. Low concentrations of pesticides can affect reproduction, longevity, population growth and behaviour, with subtle but long-lasting consequences (Lehmkuhl, 1979; Wallace et al., 1989). For example, a study which monitored the abundance of planktonic algae in pools treated with temephos, found that algal blooms consistently occurred 30 to 50 days after temephos application (Papst & Boyer, 1980). The authors attributed this phenomenon to reduced grazing pressure by zooplankton. Likewise, the reduction of blackfly predators may result in a blackfly problem worse that that before treatment began (Wallace & Hynes, 1981).

When temephos was applied at the recommended dosage for the "dry season" (0.1 ppm), the abundance of 33-44 % of the taxa present in riffles in the Orange River was reduced. These results are similar to work in West Africa which has shown that temephos applied at recommended dosages causes a 25-50 % reduction in stream invertebrates (Davies et al., 1978; Helson & West, 1978; Elouard & Jestin, 1982; de Joux 1983). Included among affected taxa in the Orange River was the important blackfly predator, *Cheumatopsyche thomasseti*.

High dosages (1.0 ppm) of temephos in an experimental canal beside the Orange River (Straussburg) affected 9 macroinvertebrates, about half of what was present (Table 15). The results are similar to other studies of temephos applied at high dosage, which report between 50 and 75 % mortality of insect larvae (Bertrand, 1976; De Joux, 1983). In West Africa, birds which ate contaminated prey died (De Joux, 1983).

Laboratory trials have indicated that certain crustacea are highly sensitive to temephos (Table 16). The 50 % lethal concentration (LC50) of temephos to fish exposed for 24 hours varies between 1.4 ppm for rainbow trout *Oncorhynchus mykiss* (Cope, 1965, in undated CYANAMID brochure), and >200 ppm for several other species (Table 16). This indicates a wide margin of safety between fish toxicity levels and the recommended dosages for blackfly control (Abban & Samman, 1980). However, when exposure times are increased to 96 hrs, the LC50 is reduced to 0.158 ppm for rainbow trout (Verschueren, 1983, in CYANAMID undated brochure), and as low as 0.04 ppm for the mummichog *Fundulus heteroclitus* (Lee & Scott, 1989). Furthermore, fishes exposed to temephos for 16 hours accumulated the toxin at concentrations over 100 times that to which they were exposed (Miles et al., 1976). *Tilapia* exposed to temephos at operational dosages (0.05 ppm for 10 minutes) developed acetylcholine inhibition, but no intoxication was noted after repeated weekly exposures (Pelissier et al., 1982; 1983, in Lêvêque et al., 1988). Likewise, fish exposed to temephos for 10 weeks, at dosages in excess of those used for blackfly control, were not

Table 15. Summary results of the effects of *B.t.i.* and temephos on riffle-dwelling macro-invertebrates in the Orange River.

Larvicide: / Dosage (ppm/10min) / Taxa	B.t.i. 1.2	B.t.i. 20	Temephos 0.05	Temephos 0.10	Temephos 1.0
Ephemeroptera					
Afronurus peringueyi	NA	-	-	-	-
Afroptilum sp.	-	-	-	-	*
Baetis glaucus	-	-	*	***	***
CAENIDAE	NA	-	-	-	-
Enthranlus elegans	-	-	-	**	-
Tricorythus discolor	NA	NA	NA	-	NA
Trichoptera					
# Amphipsyche scottae	NA	-	-	-	***
# Cheumatopsyche thomasseti	NA	NA	NA	*	***
# Ecnomus sp.	-	-	-	-	*
Orthotrichia sp.	NA	-	-	*	NA
Diptera					
# Cardiocladius ?africanus	NA	*	*	*	*
CHIRONOMINI	NA	-	-	-	-
Polypedilum sp.	-	-	-	-	*
Simulium spp.	***	***	***	***	***
Rheotanytarsus fuscus	*	***	***	**	-
Xenochironomus sp. "B"	**	-	-	-	-
Mollusca					
Burnupia capensis	-	-	NA	NA	-
Coleoptera					
ELMIDAE (larvae)	-	-	*	-	-
Annelida					
# Salifa perspicax	-	NA	-	NA	-
Bryozoa					
Plumatella sp.	NA	-	-	NA	-
Other					
NEMATODA	-	-	-	-	*
TURBELLARIA	-	NA	NA	-	-
TOTAL NUMBER OF TAXA REDUCED	3	3	5	7	9
TOTAL NUMBER NOT REDUCED	9	4	4	3	2

Data are based on the results of short-term (before-after) field trials and laboratory trials. The table lists only the taxa which were either directly affected or not affected (NA). Cross-hatching (#) indicates blackfly predators. Stars (*) indicate the degree to which taxa were affected (*P<0.05; **P<0.01; ***P<0.001).

affected (Adeney & Matthiessen, 1979). However, in West Africa fish were found to move downstream to avoid temephos. During the dry season, rivers were restricted to small pools and fish movement was restricted (Abban & Samman, 1980; 1982). This meant that in the dry season fishes may be exposed to temephos for much longer periods than in the wet season.

The effects of temephos on non-target fauna are likely to be greatest when temperatures are high and oxygen levels low. These conditions are likely to occur in quiet, shallow water. Because of time and logistic constraints, the present study was largely confined to animals living in stones-in-current biotope, where oxygen levels are usually high. Preliminary trials with the shrimp *Caridina nilotica* in the Orange River indicate that it is relatively tolerant to temephos, whereas the mayfly *Euthraulus elegans* is highly sensitive (Table 16). Snails exposed to temephos were found to have detectable levels for up to 3 weeks after exposure (Fitzpatrick & Sutherland, 1976).

The overall conclusion of the OCP's Ecological Group (an independent advisory body) is that temephos has very little long-term effect on non-target fish and invertebrate fauna (Cummins, 1985; Yaméogo et al., 1988; Letter from Dr Samba, OCP's Director, 1993). When one considers that the OCP aims to *eradicate* river blindness in man, whereas the Orange River Project aims to *control* blackflies, permissible levels of environmental contamination are expected to differ. The results of this and others studies indicate clearly that the margin of safety with temephos is very narrow. An accident or miscalculation with temephos may lead to considerable disruption to the aquatic fauna, and may affect blackfly predators. Extreme care should therefore be taken to ensure that flow determinations are accurate, and that recommended dosages are not exceeded. However, in practise, the accuracy of flow determinations and aerial applications do not have a good record. The exact concentrations applied are never known. Calibration tables are usually accurate within a limited range only, and change over time as river profiles change and reeds grow or are cleared. In 1993 the Upington gauging weir provided flow values which were roughly 4 times the actual flow in the river!

Aerial applications are also fraught with difficulties, particularly when treating braided sections of the river, such as between Keimoes and Kanoneiland (Fig. 7). There is no way of knowing what the flow in each of these channels is. The pilot has to decide, within seconds, how much larvicide to apply and what speed to travel for each channel. It takes considerable experience to make these judgments. The positions of rapids also changes considerably over time and with different flows. It was not surprising, therefore, that some channels were not treated at all during large-scale aerial treatment, while others received major overdoses.

Another problem is that spraying apparatuses have to be thoroughly washed at the end of each day's spraying to prevent corrosion. The simplest and most practical way to rid the tank of remaining larvicide (of which there is always some) is simply to dump it in the river or in the veld. To reduce the likelihood of this happening, the spray tank should be fitted with a user-friendly drainage mechanism.

Furthermore, spraying apparatuses are seldom leak-proof, and many of the parts are made of aluminium which tend to break easily. In addition, the flow gauge on the spraying apparatus is usually calibrated with water, which has a different consistency to larvicides. A further danger of aerial applications is that whereas the recommended dosages are based on a theoretical 10 minute application time, aerial application usually take less than 1 minute. This means that dosages at the point of application are more than 10 times the recommended values. de Joux (1983) considers it essential that temephos is diluted before it is loaded on the aircraft so that recommended dosages are complied with. However, a hydraulic simulation of the dispersion of a point-source pollutant indicates rapid attenuation (J. Boroto pers. comm.). High dosages are therefore restricted to a short distance (a few hundred metres) compared to the long distance (several kilometres) which is effectively treated.

The time taken for invertebrates to recover from the effects of a larvicide treatment is usually rapid. Temephos is relatively insoluble in water (1 ppm; CYANAMID brochure, undated), and like most organophosphates, dissipates rapidly in water because of microbial degradation, hydrolysis and photodegradation (Racke, 1992). Dr Kurtak (pers. comm.) suggested that the breakdown products of temephos (sulphoxides) may be more toxic than temephos itself. This phenomenon has been demonstrated for the organophosphate fenthion (Seuge & Bluzat, 1979). However, the toxicity of temephos and its breakdown products is short-lived. Preliminary tests with Orange River water showed that the half life of temephos (and the toxicity of its breakdown products) ranged between 1.6 and 3.3 days. Similar results are reported in the literature (Henry et al, 1971; Hughes et al., 1980; Lores et al, 1985).

The recovery of invertebrates following temephos treatment in rivers is usually rapid, although this has more to do with the uni-directional flow of rivers, rather than the breakdown of the product. In Canada, chironomid densities equalled or exceeded pre-treatment levels within one to three weeks, mayflies took one to four weeks, caddisflies took one to seven weeks, and stoneflies took four to five weeks to recover (Fredeen, 1977). In West Africa, chironomid numbers recovered within 21 days after temephos application at 150 times the recommended dosage (Yasuno et al., 1985). Likewise, Elouard (1975) found that no insect groups were eliminated two days after an aircraft accident which released temephos at 55 times the recommended dosage into a river.

In the Orange River, taxa which were not affected by high dosages of temephos included filter feeding bryozoa (*Plumatella* sp.) and the filter-feeding mayfly *Tricorythus discolor*. In West Africa, *Tricorythus* spp. were among the most susceptible invertebrates to temephos (Elouard & Jestin, 1982). This indicates that temephos toxicity is species-specific, and there are no general rules regarding temephos susceptibility (Muirhead-Thomson, 1978).

Table 16. Lethal concentrations (LC50) of temephos (Abate[R] 200EC) to a variety of non-target organisms, arranged in order of decreasing sensitivity.

Organism	Exposure time (hours	LC50 (ppm)	Reference
Penaeus japonicus (Shrimp)	72	0.001	Tsai, 1978
Euthraulus elegans (Ephemeroptera)	24	0.0015	This study (Preliminary result)
Penaeus aztecus (Shrimp)	48	0.0028	USDI Unpubl 1969 (in CYANAMID undated brochure)
Penaeus aztecus (Shrimp)	48	0.005	USDI Unpubl 1969 (in CYANAMID undated brochure)
Cheumatopsyche thomasseti (Trichoptera)	24	0.008	This study (Preliminary result)
Berosus afairmaiyi (Coleoptera)	72	0.008	Tsai, 1978
Baetis parvus (Ephemeroptera)	24	0.0097	Mohsen & Mulla, 1981
Penaeus duorarum (Shrimp)	48	0.01	USDI Unpubl, 1969 (in CYANAMID undated brochure)
Mugil carinatus (Pisces)	72	0.023	Tsai, 1978
Panaeus aztecus (Shrimp)	24	0.026	USDI Unpubl 1969 (in CYANAMID undated brochure)
Fundulus heteroclitus (Pisces)	96	0.04	Lee & Scott, 1989
Metapenaeus monceros (Shrimp)	72	0.045	Tsai, 1978
Panaeus monodon (Shrimp)	72	0.045	Tsai, 1978
COPEPODA	72	0.13	Tsai, 1978
Tricorythus discolor (Ephemeroptera)	24	0.15	This study (Preliminary result)
Panaeus duorarum (Shrimp)	24	0.15	USDI Unpubl 1969 (in CYANAMID undated brochure)
Salmo gairdneri (Pisces)	96	0.158	Verschueren 1983 (in Lee & Scott, 1989)
Palaemon macrodactylus (Shrimp)	96	0.249	USDI Unpubl 1969 (in CYANAMID undated brochure)
Neocaridina denticulata (Shrimp)	72	0.32	Tsai, 1978
Sarotherodon galilaea (Pisces)	96	0.47	Kpekata, 1983
Mugil cephalus (Pisces)	72	0.6	Tsai, 1978
Salmo gairdneri (Pisces)	96	1.0	Cope, 1965 (in CYANAMID undated brochure)
Hydropscyche californica (Trichoptera)	24	1.3	Mohsen & Mulla, 1981
Salmo gairdneri (Pisces)	24	1.42	Cope, 1965 (in CYANAMID undated brochure)
Salmo gairdneri (Pisces)	48	1.5	Cope, 1965 (in CYANAMID undated brochure)
Lebistes reticulatus (Pisces)	96	1.9	Kpekata, 1983
Salmo gairdneri (Pisces)	24	1.9	(CYANAMID undated brochure)
Caridina nilotica (Shrimp)	24	2.0	This study (Preliminary result)
Morone saxatilus (Pisces)	96	>>1.0	Korn & Earnest, 1974 (in CYANAMID undated)
Salvenus fontinalus (Pisces)	24	>2.0	Swabey et al., 1967
Chanos chanos (Pisces)	72	2.0-3.5	Tsai, 1978
Palaemon macrodactylus (Shrimp)	96	2.5	USDI Unpubl 1969 (CYANAMID undated brochure)
Oreochromis mossambicus (Pisces)	72	3.5	Tsai, 1978
Pimephales promelas (Pisces)	96	6.2	Swabey et al., 1967
Anguilla japonica (Pisces)	72	7.5	Tsai, 1978
Salmo gairdneri (Pisces)	96	9.6	(CYANAMID undated brochure)
Salmo gairdneri (Pisces)	48	11.5	(CYANAMID undated brochure)
Bdelloid rotifers	24	15	This study (Preliminary result)
Lepomis macrochirus (Pisces)	96	21.8	USDI Unpubl 1969 (CYANAMID undated brochure)
Lepomis cyanellis (Pisces)	48	>25	(CYANAMID undated brochure)
Salmo gairdneri (Pisces)	24	31.8	(CYANAMID undated brochure)
Lepomis macrochirus (Pisces)	24	54	USDI Unpubl 1969 (CYANAMID undated brochure)
Gambusia affinis (Pisces)	24	>200	von Windeguth & Patterson, 1966
Lepomis macrochirus (Pisces)	24	>200	von Windeguth & Patterson, 1966
Micropterus salmoides (Pisces)	24	>200	von Windeguth & Patterson, 1966

9. DISCUSSION

The results of this project have been used successfully to control blackflies along the Orange River. However, larvicides and their application are expensive, and the question which remains in most farmer's minds is how can it be done cheaply?

One possible way to reduce costs would be to produce *B.t.i.* locally. Although *B.t.i.* is easy to grow, the key to an effective product is in the formulation (Sutherland, 1990). The research to produce a product with good dispersal and ingestion properties, and a reasonable shelf-life, would be considerable. Furthermore, production runs the risk of contamination, which is a constant problem even in the most sophisticated plants. The volume of *B.t.i.* required for blackfly control along the Orange River (about 17 000 l per annum) does not warrant the expense of a local plant. However, the situation may change if *B.t.i.* is also used for mosquito and blackfly control in the subcontinent as a whole.

The work conducted during this study was used in the registration of a second *B.t.i.* product. This opened the market, and together with direct purchasing, significantly reduced the cost of *B.t.i.*, and amounted to an a saving of about R 150 000 in 1993.

Another possible way to reduce costs is to use a fixed-wing aircraft instead of a helicopter to apply larvicides. This may be possible only when the flow is high. At low-flow, fixed-wing aircraft fly too fast for accurate applications (pers. comm. with pilots and blackfly control experts). Another suggestion is to apply larvicides from bridges rather than incur the expense of aerial applications. Theoretically, bridges could be used at high flow to apply temephos between Prieska and Augrabies, but there are not enough bridges between Marksdrift and Prieska. If bridges alone were used to apply larvicides, 66 % of all rapids between Hopetown and Augrabies would be effectively dosed if the "carry" was 50 km. Therefore, bridges alone do not provide a satisfactory method of larvicide application. The construction of permanent structures has been suggested as a solution to this problem. Unless permanent structures were built at each set of rapids, they would not provide the flexibility required for efficient use of larvicides. This is because the rapids that need to be dosed vary depending on the flow. Furthermore, the distance upstream of rapids that larvicides should be applied varies with flow and larvicide formulation. The use of existing bridges and a few extra permanent structures would tend to favour the use of high dosages to increase "carry". Although "carry" does improve with increased dosage, the relation is not linear, and applications become uneconomical once the recommended dosages are exceeded

(Molloy & Jamnback, 1981). Since larvicides, and not their application, are the most expensive part of the control programme, it would be best to minimise their use. Furthermore, high dosages, particularly of temephos, are not recommended for environmental reasons (see Chapter 8). The cost of building and maintaining permanent structures that would withstand floods would be considerable, particularly considering the braided sections of the river, where the river is over 3 km wide!

Another possible method for reducing costs is to forfeit the pre- and post sampling of larvae, and simply get on with the job of applying the larvicide. This approach is extremely short-sighted because it may result in applications at times when it is not necessary. Furthermore, there would be no record of whether or not larvicides worked. There are many reasons for larvicidal failure (Table 17), and it is essential to monitor larval populations to detect such failures, and determine the reasons for failure. Once large numbers of adults have emerged, it is simply too late to control blackflies effectively.

It appears, therefore, that there are very few short cuts in controlling blackflies! However, a rough calculation shows that blackfly control still makes economic sense. In 1993 the total cost of controlling blackflies in the Orange River (using *B.t.i.* applied by helicopter) approached R 1 million. Assuming that blackflies are a nuisance up to 50 km on either side of the Orange River, the estimated cost per stock unit can be determined. In 1993 the river was treated between Hopetown and Augrabies Falls, protecting an area of roughly 50 000 km^2 (taking river bends into account). The carrying capacity of this area is estimated at 5 to 6 ha per sheep, which means that approximately one million sheep enjoyed a blackfly free year. In other words, blackfly control was achieved at an approximate cost of R 1 per sheep per annum. The Northern Cape Agricultural Union found that lambing percentages in 1993/94 were exceptionally high, and one farmer estimated that annual losses due to blackflies during pre-control years exceeded R 30 000!

Although blackfly control appears expensive, the costs of no control are orders of magnitude greater (up to R 33 million, estimated in 1990). At current prices (roughly R 46/l for temephos and R 28/l for *B.t.i.*), temephos is about 1.6 times more expensive per litre. However, temephos carries between 1.5 and 4.4 times further than *B.t.i.*, depending on flow (Table 14). When the river is low (<70 m^3/s) the cost of treating a section of river is roughly the same for the two products. As flows increase, *B.t.i.* becomes increasingly expensive compared to temephos, and may be as much as 3 times the cost.

Not only are larvicides expensive, but they incur environmental costs. The interval between larvicide applications recommended for blackfly control is shorter than that required for the recovery of most non-target fauna. A series of larvicide applications, particularly in an isolated river, with no middle reach tributaries, such as the Orange River, may therefore be detrimental to susceptible species with poor re-colonisation attributes. Repeated application of the same larvicide, particularly temephos, may therefore lead to major changes in the ecosystem and possible damage to ecological processes.

Furthermore, each application increases the chances of resistance developing. Methods to reduce the development of resistance include the use of different larvicides in different areas, the temporal alternation of larvicides, and the provision of temporal and spatial refugia (Tabashnik, 1994).

Therefore, the best option for blackfly control (both financially and environmentally), is to minimise the number treatments. Timing of treatments should be stretched out as far as possible, although not too far as to allow large numbers of adult females to emerge.

The success or failure of insect control programmes worldwide often hinges on public perceptions (Service pers. comm.). In South Africa, public awareness of environmental issues is growing fast, and it is increasingly important for the public to be kept informed about the control programme. This means that the people applying the larvicides should be informed about the effects of the larvicides and safety precautions, so that questions of observers may be answered.

Table 17. Possible reasons for larvicidal failure, and suggestions for remedy.

* Application too near the breeding site. Insufficient mixing and/ or larvicide passes over the larvae too fast to allow them time to ingest. Apply larvicide further upstream.
* Application too far upstream. Larvicides settle out of suspension before getting to the breeding site. Check for deep pools or the presence of aquatic vegetation between application point and breeding site. Apply larvicide closer to breeding site.
* Time taken to apply larvicide too long. Larvicide becomes too dilute to be effective. Apply larvicide faster.
* Time taken to apply larvicide too short. The larvicide passes over the larvae too fast to allow them time to ingest. Slow down.
* Sampling site badly situated. It may be that the larvicide did in fact work, but not at the place at which larval numbers were monitored. It is therefore important to monitor larval populations right across the river, including both banks.
* River discharge was underestimated. Therefore dosage was too dilute. Check calculations.
* Water temperature too cold (<10^{0}C). Larvae feed slowly at cold temperatures. They therefore ingest less larvicide. Consider increasing dosage.
* Water too turbid (from silt) or green (from planktonic algae). Consider increasing dosage.
* Water was polluted. Check for municipal sewage works or industrial effluent. Check levels of chloride. Values > 50 mg/l may affect B.t.i. efficacy.
* Formulation was faulty. Check how the product behaves in water. It should disperse evenly throughout the water column. If it forms clumps which rapidly drop to the bottom, or remains in a thin layer on the surface of the water, something is wrong with the formulation. Consult with supplier.
* Product was too old. Check date of production. Perhaps it has been standing too long. Try fresh product.

10. CONCLUSIONS AND RECOMMENDATIONS

Based on the results of this project, the following PROTOCOL for the control of blackflies in the middle Orange River is recommended.

10.1 When to apply

- Blackfly outbreaks occur every spring (Aug-Nov) and sometimes in autumn (Mar-May). Therefore, control of the spring outbreak can be planned well in advance, but a flexible protocol is required to control outbreaks in autumn. Severe outbreaks of *S. chutteri* may be anticipated after a long, cold winter with high flow (>144 m^3/s).

- The first treatment of the "spring" season should take place in the last week or two of July. Timing of successive treatments depends on water temperature, as indicated in Table 10 and Table 11. If water temperature in the last week of July is 12^{0}C or less, the second treatment should take place in the last week of August, 32 days after the first treatment. The third treatment should take place in mid-September, 18 days after the second treatment. The fourth and final treatment should take place in the last week of September, 13 days after the third treatment. If water temperature in July exceeds 12^{0}C, treatment intervals should be reduced.

- The first treatment of the "autumn" season depends on river conditions, but is likely to occur in mid-March. Subsequent treatments should take place at 9 to 14 day intervals, as indicated in Table 11. Six treatments should be sufficient to prevent an outbreak in autumn.

- If the recommended treatment intervals cannot be adhered to, a shorter interval should be used. Longer treatment intervals should be avoided.

- Treatments may be affected by algal blooms, which are likely to occur between February and March if the water is clear (TSS < 40 mg/l; Secchi depth > 35 cm).

10.2 Where to apply

- Between 98 and 134 waterfalls, rapids and riffles have been identified as blackfly breeding sites between Hopetown and Augrabies Falls. The number depends on the flow. Not all these rapids will need to be treated every time. The sites to be treated will depend on the expected "carry", as indicated in Table 14. "Carry" is highly variable, and depends on the presence of pools, the river discharge, the product used, dosage applied, and the river conditions.

- IT IS PREFERABLE TO TREAT MORE RAPIDS AT A LOWER DOSAGE, THAN TO TREAT A FEW RAPIDS AT HIGH DOSAGE.

- Areas should be left untreated to reduce the chances of resistance developing, and provide refuges from which recolonisation can occur.

10.3 What to apply

- *B.t.i.* MUST ALWAYS BE USED IN PREFERENCE TO TEMEPHOS. Reasons for this include the effects of temephos on "non-target" organisms, particularly blackfly predators, and the possible development of larvicidal resistance. TEMEPHOS SHOULD BE USED ONLY IF THE FLOW EXCEEDS 200 m^3/s, OR IF THE NUMBER OF ALGAL CELLS EXCEEDS 1500/ml, OR IF THE CONCENTRATION OF TOTAL SUSPENDED SOLIDS EXCEEDS 150 mg/l (Kurtak *pers. comm.*), OR IF THE SECCHI DEPTH READING IS LESS THAN 12 cm.

10.4 How to apply

- The distance upstream of rapids that larvicides should be applied varies with flow, the site, as well as the larvicide formulation. Larvicides should not be applied upstream of deep pools, weirs or aquatic vegetation. Buoyant larvicides (such as current formulations of temephos), should be applied much further upstream (at least double the distance) compared to less buoyant larvicides (such as current formulations of *B.t.i.*). As a general rule, larvicides should be applied about 50 m upstream at low-flow ($\leq$59 m^3/s), 100 to 300 m upstream at moderate-flow (60-143 m^3/s) and 400 m upstream at high-flow (>144 m^3/s).

- *Treatments must always start from downstream and work* upstream so as to prevent interference with water that has already been treated.

- Care should be taken when applying larvicides, especially temephos, under windy conditions.

- THE AMOUNT OF LARVICIDE APPLIED PER SITE SHOULD TAKE INTO ACCOUNT THE DISTANCE BETWEEN RAPIDS, AND ONLY SUFFICIENT LARVICIDE TO TREAT DOWNSTREAM RAPIDS SHOULD BE APPLIED.

- TEMEPHOS MUST NOT BE APPLIED AT DOSAGES EXCEEDING 0.1 PPM/10 MINUTES. OVERDOSING WITH TEMEPHOS SHOULD BE STRICTLY AVOIDED. Temephos must not be applied where the river divides into multiple (>2) channels.

- Care should be taken to apply larvicides, especially *B.t.i.*, from bank to bank.

- *B.t.i.* SHOULD BE SHAKEN WELL PRIOR TO USE. However, excess agitation of some formulations traps air and turns the larvicide into a thick consistency which is difficult to apply. It is therefore important to read the application instructions.

- Extra temephos which remains in the spray tank at the end of a days spraying must not be dumped.

10.5 Dosages

- Current formulations of *B.t.i.* are applied at a rate of 7.2 l per 10 m^3/s, equivalent to 1.2 ppm when applied over 10 minutes (see review by Knutti & Beck, 1987). However, dosages of *B.t.i.* may be doubled when the concentration of TSS exceeds 150 mg/l, or when the number of planktonic algal cells exceeds 1500/ml.

- In West Africa temephos (Abate^R 200EC) is applied at a rate of 1.5 l per 10 m^3/s in the wet season (equivalent to 0.05 ppm when applied over 10 minutes), and 3 l per 10 m^3/s (or 0.1 ppm) in the dry season. (Dosages are well within the safety limits (1.0 ppm) prescribed for drinking water by the WHO.) Adequate control in the Orange River was obtained using the lower (0.05 ppm) dosage.

10.6 Monitoring

- In the week preceding the first treatment of a season, the river should be surveyed to ensure that (1) larval populations of *S. chutteri* are sufficiently high to warrant treatment, and (2) the water conditions are suitable for the use of *B.t.i.*.

- Larval numbers should be monitored at at least 5 % of the treated breeding sites before and after each treatment (i.e., five sites).

- The following variables should be recorded for each treatment: (1) Water temperature, (2) The concentration of total suspended solids (or Secchi depth) (3) The concentration and composition of planktonic algae, (4) The blackfly species present and (5) blackfly larval and pupal abundance.

- Adult numbers should continue to be monitored on a daily or weekly basis throughout the year by interested and concerned farmers. Complaints of blackflies should be directed through the Northern Cape Agricultural Union Blackfly Control Committee or the Directorate of Resource

Conservation.

- The construction of a sloping embankment which provides constant hydraulic conditions at all river flows, and which can be used to obtain reliable estimates of blackfly numbers, should be considered. The embankment would be used to monitor larval abundance throughout the year, and warn of a pending outbreak. However, careful monitoring and calibration of larval and pupal densities against blackfly densities in the river would need to be carried out.

10.7 General

- The protocol for blackfly control must remain flexible. Contact with the WHO/OCP in West Africa and other control programmes worldwide should be maintained, so that recommendations and new developments, such as the use of *Clostridium bifermentans* as a blackfly control agent, are incorporated into the Orange River Programme.

- Each new batch of larvicides should be tested in small-scale field trials prior to large-scale treatment to ensure that *it works. (Small changes to the formulation can* result in large changes in efficacy.)

- The dispersal properties of each new batch of larvicides should be examined. This can be done by dropping 1 ml of larvicide from a syringe held 20 cm height, into a glass containing 1 l of river water. The time taken for the larvicide to reach the bottom of the container, and disperse evenly throughout the water is a simple and effective method of establishing its dispersal properties (Kurtak *pers. comm.*). Larvicides with low density remain on the surface of the water, and application sites therefore need to be adjusted accordingly.

- Good and frequent communication between the Blackfly Control Programme and the Department of Water Affairs is essential for effective blackfly control. River flow should be as stable as possible during treatments. Arrangements should therefore be made that releases from P.K. le Roux Dam should be stabilised ten days prior to treatment.

- The public must be kept informed about the control programme through the local newspaper ("Die Gemsbok") and farmer's unions, and the results of all applications must be transparent.

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11. FUTURE RESEARCH

There is an urgent need for a more sophisticated system of deciding whether or not to apply larvicides. This was highlighted in February 1993, when a flush of water in the Orange River triggered the hatching of *S. chutteri* eggs. An outbreak was predicted based on the high numbers of larvae at several sites along the river. However, the flow dropped and the outbreak never materialised. Had it not been that the new helicopter could not be commissioned in time, the river would have been treated unnecessarily, at a cost of between R 100 000 and R 300 000. Future economic limitations may require that farmers arrange for, and pay for, control measures. Therefore, the correct and minimal applications of larvicides will be even more critical than at present.

There are still several gaps in our understanding of the blackfly problem. These are:

- The link between flow levels and habitat availability. (This information would be useful to artificially manipulate flows so as to minimise the need for larvicidal treatments.)

- The link between larval numbers and adult annoyance.

- The link between adult annoyance and financial losses due to blackflies. (Sometimes a single fly is enough to prevent sheep from feeding!)

- The role that planktonic algae plays in blackfly population dynamics.

- The role that eggs play in blackfly population dynamics.

- The role that naturally occuring predators play in regulating *S. chutteri* populations.

- The medium- to long-term effects of temephos on non-target organisms.

The decision to apply larvicides is both complex and expensive. By understanding the variables which govern blackfly outbreaks, and the likely consequences (including costs) of the two options (to spray or not to spray), future research should aim to provide a framework for minimising the number of treatments, and therefore the costs (both financial and environmental), of blackfly control.

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13. APPENDICES

Appendix A. Specifications of sites in the Orange River visited during large-scale river surveys in summer (18-25.02.92) and winter (6-22.07.92). "Distance" refers to the downstream river distance from P.K. le Roux Dam, with negative numbers indicating upstream distance. Dotted lines indicate the positions of impoundments.

Site	Grid		Altitude	Distance	
	South	East	(m)	(km)	
1. Walaza	30°24''	27°20''	1400	-456	
- - - - - - - - - - - -	- - - -	- - - -	- - - -	- - - -	Hendrik Verwoerd
2. HF2: 2.5 km below Verwoerd D.	30°37''	25°28''	1200	-133	
3. Serfontein Bridge	30°30''	25°12''	1189	-91	
- - - - - - - - - - - -	- - - -	- - - -	- - - -	- - - -	P. K. Le Roux
4. PK: Directly Below PK.	29°59'32"	24°43'26"	1184	0.7	
5. Havenga Bridge	29°54'45"	24°38'13"	1167	18.5	
6. Orania	29°47'21"	24°24'37"	1153	44.9	
7. Marksdrift	29°09'40"	23°41'39"	1029	199.6	
8. Prieska Bridge	29°39'26"	22°44'45"	985	379.4	
9. G2 (Gifkloof 2)	28°26'13"	21°24'06"	852	638.5	
10. Onseepkans	28°44'13"	19°18'21"	360	916.6	
11. Vioolsdrift	28°41'51"	17°30'00"	185	1138.6	

Appendix B. Specifications of impoundments and large weirs in the middle and lower Orange River. Data supplied by the Department of Water Affairs and Forestry, Pretoria.

Dams:	Verwoerd	P.K. Le Roux	Boegoeberg	Neusberg
Completed	1971	1977	1929	1993
Area (km^2)	359	138	6.9	1.1
Capacity $10^6 m^3$	5,670	3,236	21	1.8
Catchment area (km^2)	70,749	89,842	342,952	368,830
Mean depth (m)	16.3	23.3	2.7	-
Maximum depth (m)	64	73	-	-
Height of wall (m)	88	107	10	4
Length of wall (m)	914	765	622	860

Appendix C. Data collected at various sites (G1 to G4) downstream of Gifkloof Weir every Monday between July 1991 and June 1994. "Temp" refers to the weekly maximum-minumum water temperature (in ^{0}C). "Flow" refers to the daily average flow recorded at Boegoeberg Dam (D7H008) by the Department of Water Affairs, Upington. "TSS" refers to the concentration of total suspended solids. "Benth" refers to the percentage cover of benthic (epilithic) algae. The abundance of blackfly larvae and pupae are shown as the median values recorded on 30 rocks, measured on a 10 point scale (see Fig. 23-26). The 95% confidence limits of the median are given in brackets.

Date	Site	Temp. min-max	Flow (m^3/s)	TSS (mg/l)	Benth (%)	Larvae (1-10)	Pupae (1-10)	Comments
29 Jul 91	G2	---	102	59	25-50	7(5-7)	-	B.t.i. applied at Gifkloof
5 Aug 91	G2	10-12	91	-	75-100	2(1-2)	-	
12 Aug 91	G1	10-13	99	56	75-100	2(1-2)	-	
19 Aug 91	G2	11-15	93	66	75-100	2(1-4)	-	
26 Aug 91	G2	12-15	99	53	75-100	6(5-7)	-	
2 Sep 91	G2	13-15	102	57	75-100	7(6-7)	-	Adult blackflies common
9 Sep 91	G2	14-16	87	61	75-100	5(4-7)	-	
16 Sep 91	G2	14-18	131	30	25-50	7(7-8)	-	
23 Sep 91	G2	16-19	84	103	25-50	-	-	
30 Sep 91	G2	17-21	110	62	5-25	-	-	
7 Oct 91	G2	19-21	91	53	5-25	8(8-9)	-	
14 Oct 91	G1	20-23	93	39	5-25	8(7-8)	-	
21 Oct 91	G2	20-23	117	76	5-25	8(8-9)	-	
28 Oct 91	G1	---	379	144	1-10	-	-	Thermometer swept away
4 Nov 91	-	---	267	-	-	-	-	
11 Nov 91	G1	---	294	118	1-10	6(5-7)	-	
18 Nov 91	G2	22-26	389	77	1-10	7(7-8)	-	
25 Nov 91	G2	21-25	289	86	1-10	1(1-3)	-	*B.t.i.* applied at Gifkloof
2 Dec 91	G1	21-26	161	76	1-10	8(8-8)	-	
9 Dec 91	G1	22-26	129	80	1-10	8(6-8)	-	
17 Dec 91	G1	---	214	-	-	-	-	
23 Dec 91	G1	---	253	-	-	-	-	
30 Dec 91	G1	22-26	249	73	5-25	8(8-9)	-	
6 Jan 92	G1	24-26	216	83	1-10	7(6-7)	-	
13 Jan 92	G1	25-29	102	74	5-25	9(8-9)	5(3-5)	
20 Jan 92	G1	25-27	169	58	5-25	6(5-7)	3(2-4)	
27 Jan 92	G1	25-28	180	78	5-25	7(6-8)	6(4-7)	
3 Feb 92	G1	25-29	174	70	5-25	7(6-8)	4(2-5)	
10 Feb 92	G2	25-27	154	79	5-25	8(7-9)	6(4-7)	
17 Feb 92	G2	---	159	76	25-50	6(5-7)	5(4-6)	Thermometer washed away
24 Feb 92	G2	25-27	102	55	25-50	7(6-8)	3(3-5)	
2 Mar 92	G2	21-25	117	49	25-50	6(4-7)	4(3-5)	
9 Mar 92	G2	20-25	115	42	75-100	7(4-7)	5(3-6)	
16 Mar 92	G2	24-26	84	46	25-50	7(6-7)	5(4-6)	Construction of weir: scour
23 Mar 92	G2	22-25	87	43	5-25	5(5-6)	3(2-5)	
30 Mar 92	G2	21-25	84	55	5-25	6(5-7)	3(3-4)	
6 Apr 92	G2	21-24	89	59	5-25	7(6-8)	4(3-4)	
13 Apr 92	G2	19-25	110	51	5-25	8(7-8)	5(5-6)	
20 Apr 92	G2	19-22	115	58	5-25	7(6-8)	5(4-5)	
27 Apr 92	G2	17-22	93	51	5-25	8(7-8)	5(5-6)	
4 May 92	G2	14-21	85	51	5-25	8(7-8)	5(5-6)	
11 May 92	G2	14-16	104	57	5-25	8(8-9)	8(7-8)	Adult blackflies abundant
18 May 92	G2	15-17	127	61	5-25	9(8-9)	5(5-6)	Empty pupae abundant
25 May 92	G2	13-16	82	50	5-25	9(9-10)	9(9-9)	Drop in flow
1 Jun 92	G2	11-14	89	60	5-25	8(8-9)	8(7-8)	
8 Jun 92	G2	10-13	89	54	25-50	9(9-10)	8(7-8)	
15 Jun 92	G2	10-12	91	52	25-50	8(8-9)	6(5-7)	

Appendix C. continued....

Date	Site	Temp. min-max	Flow (m^3/s)	TSS (mg/l)	Benth (%)	Larvae (1-10)	Pupae (1-10)	Comments
22 Jun 92	G2	10-12	99	48	25-50	9(8-9)	5(4-6)	Empty pupae abundant
29 Jun 92	G2	9-11	97	44	5-25	9(8-9)	5(3-5)	
6 Jul 92	G2	9-11	91	34	25-50	8(8-9)	2(1-2)	
13 Jul 92	G2	8-11	74	42	75-100	10(9-10)	2(1-2)	
20 Jul 92	G2	10-13	78	31	75-100	10(9-10)	4(3-6)	
27 Jul 92	G2	11-13	89	47	25-50	10(9-10)	7(5-8)	
3 Aug 92	G2	10-14	85	41	25-50	10(9-10)	9(6-10)	
10 Aug 92	G2	10-13	72	46	75-100	1(1-1)	1(1-2)	Temephos applied at Gifkloof
17 Aug 92	G2	12-14	74	43	75-100	6(6-7)	7(6-7)	
24 Aug 92	G2	9-15	67	29	75-100	7(7-8)	1(1-1)	
31 Aug 92	G2	12-17	71	29	25-50	8(8-8)	1(1-1)	Water green, mainly diatoms
7 Sep 92	G2	15-18	59	24	25-50	9(9-9)	7(7-8)	
14 Sep 92	G2	15-20	59	33	25-50	9(9-9)	9(8-9)	High mermithid infections
21 Sep 92	G2	18-21	59	26	25-50	9(8-9)	9(9-9)	
28 Sep 92	G2	15-20	59	29	25-50	8(8-8)	6(6-7)	
5 Oct 92	G2	18-23	56	29	25-50	8(8-9)	4(4-5)	
12 Oct 92	G2	19-23	56	37	25-50	9(8-9)	8(6-8)	
19 Oct 92	G2	19-24	59	44	25-50	9(8-9)	8(8-9)	
26 Oct 92	G2	18-21	58	46	25-50	8(7-8)	7(6-7)	
2 Nov 92	G2	19-22	56	43	25-50	8(8-9)	8(7-8)	
9 Nov 92	G2	20-25	54	45	25-50	9(8-9)	8(7-8)	
16 Nov 92	G2	21-25	59	44	25-50	8(8-9)	8(7-8)	
23 Nov 92	G2	21-23	61	44	25-50	8(8-9)	7(6-8)	
30 Nov 92	G2	21-23	108	44	25-50	8(8-9)	7(6-7)	
7 Dec 92	G2	22-27	63	44	25-50	8(7-8)	6(5-7)	
14 Dec 92	G2	23-25	61	38	25-50	7(7-8)	5(4-6)	
21 Dec 92	G2	24-26	59	35	25-50	7(6-7)	5(4-5)	
28 Dec 92	G2	---	54	--	-	-	-	
4 Jan 93	G2	22-27	56	31	25-50	6(6-7)	5(4-5)	Thermometer left for 18 days
11 Jan 93	G2	25-29	44	22	25-50	5(5-7)	4(3-5)	
18 Jan 93	G2	25-28	64	23	5-25	5(4-5)	3(2-3)	
25 Jan 93	G2	25-27	68	34	25-50	6(5-7)	4(4-5)	
1 Feb 93	G2	23-26	50	31	25-50	7(6-7)	5(5-6)	
8 Feb 93	G2	24-27	40	40	25-50	8(7-8)	5(5-5)	
15 Feb 93	G2	24-27	51	31	25-50	8(7-8)	6(6-7)	
22 Feb 93	G2	24-27	56	37	10	7(6-7)	5(4-6)	
1 Mar 93	G2	24-28	51	34	10	7(6-8)	6(5-7)	Start planktonic algal bloom
8 Mar 93	G2	25-27	39	36	1	5(5-7)	5(4-6)	
15 Mar 93	G2	24-26	29	26	1	4(3-6)	1(1-1)	Height of algal bloom
22 Mar 93	G2	21-26	29	25	10	2(2-4)	1(1-1)	
29 Mar 93	G2	23-24	28	25	0	1(1-2)	1(1-1)	
5 Apr 93	G2	22-24	73	20	1	2(1-5)	1(1-1)	
12 Apr 93	G3	15-23	27	29	1	4(3-5)	2(2-2)	Boegoeberg drained
19 Apr 93	G3	---	38	16	1	6(6-6)	2(2-3)	
26 Apr 93	G3	20-23	33	21	10	3(2-5)	2(2-3)	
3 May 93	G3	19-21	30	18	10	3(2-4)	2(1-3)	
10 May 93	G3	16-18	26	23	10	4(3-4)	2(2-3)	Farmers start complaining
17 May 93	G3	15-18	32	14	10	3(2-3)	3(2-3)	
24 May 93	G3	14-16	33	17	10	4(3-5)	3(2-3)	
31 May 93	G3	12-14	15	11	25	4(3-4)	3(2-3)	
7 Jun 93	G3	10-12	14	6	50	5(3-5)	4(3-5)	
14 Jun 93	G3	10-13	15	5	50	5(4-6)	3(3-5)	
21 Jun 93	G3	12-14	16	21	50	5(4-5)	3(3-4)	
28 Jun 93	G3	---	20	--	-	-	-	
5 Jul 93	G3	9-14	16	15	50	6(5-6)	3(2-3)	Benthic algae senescent
12 Jul 93	G3	12-15	17	14	50	6(5-6)	4(3-4)	
19 Jul 93	G3	11-15	15	10	50	5(5-5)	3(3-4)	
26 Jul 93	G3	12-16	16	12	50	6(5-6)	4(3-4)	
2 Aug 93	G3	13-15	15	8	25	5(5-6)	4(3-4)	Benthic algae senescent
9 Aug 93	G3	11-15	16	7	10	6(5-6)	4(4-4)	
16 Aug 93	G4	11-15	14	10	50	8(8-8)	7(7-8)	
23 Aug 93	G4	14-16	14	10	75	8(7-8)	6(5-7)	
30 Aug 93	G4	14-18	15	12	75	8(7-8)	5(5-6)	

Appendix C. continued....

Date	Site	Temp. min-max	Flow (m^3/s)	TSS (mg/l)	Benth (%)	Larvae (1-10)	Pupae (1-10)	Comments
6 Sep 93	G4	16-20	15	12	50	7(7-8)	4(4-5)	
13 Sep 93	G4	17-20	15	11	25	8(7-9)	5(4-6)	
20 Sep 93	G4	17-22	15	16	25	8(7-8)	3(2-4)	
27 Sep 93	G4	16-21	15	13	10	7(7-7)	3(2-4)	
4 Oct 93	G4	17-23	15	15	5	8(7-8)	5(4-6)	
11 Oct 93	G4	-----	29	9	0	1(1-1)	1(1-1)	Thermometer washed away
18 Oct 93	G4	18-24	21	13	10	6(6-7)	5(3-6)	
25 Oct 93	G4	21-25	26	18	10	6(4-6)	3(2-3)	
1 Nov 93	G4	24-26	23	29	10	6(6-7)	4(4-4)	
8 Nov 93	G4	24-26	19	25	10	5(5-6)	3(3-4)	
15 Nov 93	G4	21-25	40	29	25	5(5-5)	4(3-4)	
22 Nov 93	G3	23-26	42	43	5	4(3-4)	3(2-3)	Microsporidia abundant
29 Nov 93	G3	23-24	53	--	5	4(3-4)	3(2-4)	
6 Dec 93	G3	22-25	41	30	5	5(5-6)	3(2-4)	
12 Dec 93	G5	24-26	36	38	25	5(4-5)	4(4-5)	
20 Dec 93	--	---	38	--	-	-	-	
27 Dec 93	--	---	48	--	-	-	-	
3 Jan 94	G3	22-27	51	54	5	3(3-4)	2(2-2)	
10 Jan 94	G3	24-26	62	--	10	3(2-3)	2(2-3)	
17 Jan 94	G3	24-26	107	--	10	3(2-4)	2(1-2)	
24 Jan 94	G3	---	59	78	10	5(2-6)	2(2-2)	
31 Jan 94	G3	26-29	55	66	25	5(4-5)	4(3-5)	
7 Feb 94	G3	---	74	85	25	2(1-4)	2(1-4)	*Lemna gibba* present
14 Feb 94	G3	26-28	150	120	1	2(2-4)	3(2-3)	Adult *S. damnosum* biting
21 Feb 94	G3	26-29	87	67	5	4(3-5)	3(2-3)	Microsporidia abundant
28 Feb 94	G3	26-28	72	57	10	7(6-8)	4(3-5)	
7 Mar 94	G3	24-27	72	65	10	5(4-6)	4(3-5)	Local rain
14 Mar 94	G3	25-28	76	41	25	3(3-4)	3(3-4)	
21 Mar 94	G3	23-25	62	41	50	5(4-6)	3(2-4)	
28 Mar 94	G3	22-26	57	45	50	4(2-4)	2(2-3)	Pupal fungal infections
4 Apr 94	G3	23-24	62	..	50	4(3-6)	2(2-3)	
11 Apr 94	G3	22-24	54	..	25	4(3-5)	2(1-3)	
18 Apr 94	G3	21-24	64	..	50	5(2-6)	2(2-3)	
25 Apr 94	G3	-----	64	..	50	2(1-2)	2(1-3)	
2 May 94	G3	17-21	57	..	25	4(3-5)	3(2-3)	
9 May 94	G3	15-17	71	..	50	5(3-5)	4(3-4)	
16 May 94	G3	16-17	72	..	50	6(5-7)	3(2-4)	
23 May 94	G3	16-18	67	..	75	6(5-7)	4(4-5)	
30 May 94	G3	11-17	202	..	75	5(4-6)	2(1-2)	Sudden increase in flow
6 Jun 94	G2	12-12	256	..	1	7(6-7)	4(4-5)	
13 Jun 94	G2	11-11	256	..	1	4(3-5)	1(1-2)	
20 Jun 94	G2	10-11	...	..	1	5	2	