PILOT STUDY INVESTIGATING THE CURRENT STATUS AND CHANGES IN THE PEST BLACKFLY (DIPTERA: SIMULIIDAE) PROBLEM ON THE ORANGE RIVER

Report to the
Water Research Commission

by

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The publication of this report emanates from a project titled *Pilot study on investigating the current status and changes in the pest blackfly (Diptera: Simuliidae) problem on the Orange River* (WRC Project No. K8/1004).

EXECUTIVE SUMMARY

Blackfly along the middle and lower reaches of the Orange River are major pests of livestock and labour-intensive agriculture, with losses to the industry calculated to exceed R300 million per annum based on current meat prices. The problem is attributed to winter high flows, with the main pest species being *Simulium chutteri* Lewis although there are times when *S. damnosum* s.l. Theobald and *S. impukane* de Meillon are problematic. During 2011, blackfly outbreaks in the middle and lower reaches of the Orange River were noted to have worsened. It was proposed that current reduced success in controlling *S. chutteri* using larvicides could be one of the reasons for this. Alternatively, or additionally, outbreaks may have been due to other species of blackfly not being targeted for control, particularly *S. impukane*. The aim of this study was to investigate the likelihoods of these competing reasons, and to propose control options.

A weight-of-evidence approach was used to assess the most likely causes of the recent problems. The approach included a comprehensive literature review of the hydraulic preferences of both species, complemented by hydrological analyses of flow patterns from relevant gauging weirs along the middle Orange River to assess available habitat. A field survey of sites covering fast- and slower-flower habitats in single-channel and anastomosing reaches of the Orange River between Upington and the downstream town of Keimoes was undertaken during November 2012. All data were related back to the classification of Palmer and Craig (2000) which classifies blackfly larvae based on seston concentration and flow velocity preferences. This was used as a predictive framework for assessing what the most likely species responsible for the outbreaks was likely to be based on flow and habitat conditions. A third dimension of thermal preferences was considered, based on preliminary laboratory experiments to establish thermal thresholds (LT_{50} values). This was included to test the hypothesis that a species preferring higher flows was more likely to have a lower LT_{50} than a species which occurred in lower flow habitats.

A total of six species of *Simulium* were recorded: *S. adersi*; *S. chutteri*; *S. damnosum*; *S. mcmahoni*; *S. medusaeforme*; *S. ruficorne*, with no individuals of *S. impukane* found. *S. chutteri* was the most abundant and widespread species, while *S. ruficorne* was restricted to the slow-flowing, highly saline agricultural return flow channels. Notably, this was the first time that *S. medusaeforme* has been recorded from the Orange River (Palmer, 2012,

pers. comm.). The blackfly species sampled showed distinct flow velocity preferences, with *S. chutteri* being the only species preferring flows > 1 ms^{-1}, while the remaining species showed preferences for lower flows across a spectrum from 0.35-0.68 ms^{-1}. Analyses of flow data confirmed that flow conditions for a species such as *S. impukane* are only favourable for 1% of the time for flows of up to 30 m^3s^{-1}, although this may be higher in the anastomosed sections.

Based on the combined evidence, it is unlikely that the outbreaks experienced during 2011 were the result of another species of blackfly additional to *S. chutteri*. Hydrologically, it is an anomaly that *S. impukane* is a problem in the Orange River. Given its larval habitat preferences, it was noted that there should always be some habitat for *S. impukane* along certain reaches of the Orange River. Optimal habitat conditions would be during low-flow periods of clear water. It is therefore most likely that the main cause of the blackfly problem remains *S. chutteri*, where sustained high flow volumes and turbidity levels favour this species over the other species of blackfly. However, during periods of lower flow and lower turbidity, other species of blackfly may be favoured and contribute towards periodic outbreaks. Additionally, anasotomosing reaches of the Orange River, such as near Keimoes, which are difficult to apply larvicides to because of limited downstream carry, may act as reservoirs of various species of blackfly. This may contribute towards periodic outbreaks of pest blackfly, caused by multiple blackfly species including *S. damnosum* and *S. impukane*.

Ongoing monitoring is recommended, where species are recorded, as well as turbidity levels. What this study has highlighted is that the best avenues for improved blackfly control should focus on the following two areas:

- Management issues around the control programme itself, as previously highlighted by Palmer et al. (2007)
- Hydraulic studies linked to investigations of downstream carry of larvicides in the anastomosing sections, which are likely to be the least well controlled areas on the middle Orange River. However, while it is unfortunate that these sections are associated with human settlement areas, it is also important to bear in mind that residual populations of different species of blackfly should be maintained in the range river as parts of its natural ecological functioning, and that the anastomosing sections could be serving a role as refugia.

ACKNOWLEDGEMENTS

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The following individuals are sincerely thanked for their various contributions to the project:

Name	Affiliation	Contribution
Dr Gerhard Backeberg	WRC	Project initiation discussions
Mr Ian Garden	Philagro	Project facilitation
Mr Hoffie Joubert	Northern Cape Farmer	Project initiation discussions
Ms. Nthabiseng Kgotla	NDA, Upington Office	Collection of live material
Mr John Mansfield	Philagro	Project facilitation
Mr Jeremy Naidoo	3rd-year Hydrology student, UKZN	Hydrology and water quality analyses
Mr Thabani Ndlovu	Hydrology Honours student, UKZN	Hydrology and water quality analyses
Mr Vere Ross-Gillespie	PhD student, UCT	Thermal experiments
Time Freight Couriers		Transport of live material

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INTRODUCTION: CHARACTERISATION OF CURRENT CONTROL PROGRAMME AND IDENTIFICATION OF GAPS

Background to general problem

Increased demands on water resources in South Africa over the past 40 years have resulted in dams being built and inter-basin transfers (IBTs) occurring to make water more easily accessible and readily available on a sustainable basis (de Moor 2002). However, changes in flows from such actions result in changes to ecological conditions and consequent changes in aquatic biota and community composition of species (O'Keeffe and de Moor 1988).

One such example of ecological consequences is in the Orange River, where one group of aquatic macroinvertebrates (blackfly – Diptera: Simuliidae) has become a pest in response to altered flows. The blackfly problem in the Orange River began from the 1970s following completion of the Gariep and Vanderkloof Dams in the mid-reaches (Palmer 1998). Myburgh (2003) demonstrated the links between winter water temperatures and physiological responses of larval blackfly, which underpin the higher fecundity of the spring population and hence the propensity for spring outbreaks if not controlled during winter. The problem is attributed to winter high flows, with the main pest species being *Simulium chutteri* Lewis although there are times when *S. damnosum* s.l. Theobald and *S. impukane* de Meillon are problematic (Palmer and Rivers-Moore 2008).

Blackfly along the middle and lower reaches of the Orange River are major pests of livestock and labour-intensive agriculture (Palmer 1997). The outbreaks have a major impact on sheep farming and grape industries, with cost estimates ranging from R67m (Palmer et al 2007) to R88m (Palmer 1997), to more recent surveys of R133 million per annum in the agricultural sector alone (2004 figures at R20/kg for mutton, which would equate to *ca*. R333 million at current meat prices of R50/kg). Outbreaks of blackfly continue to cause significant economic losses to the tourism, labour and agricultural sectors. Agricultural impacts occur through loss in conception, stock mortalities and loss in body weight gain.

The blackfly control programme was initiated by the Department of Agriculture following on from an extensive study by Palmer (1997). The Orange River Control programme focuses on the middle and lower reaches, extending over approximately 850 km of river – 148 blackfly breeding sites, with an average of one site every 5.7 km (Rivers-Moore et al.

2008a). While an integrated control programme was strongly recommended, focussing on the low-flow July period (when 70% of flows are < 100 m^3s^{-1}), the main control approach remains through larvicide applications, (Palmer 1997; Rivers-Moore et al. 2008b). Aerial applications of larvicides have been applied since 1991 to control larval blackfly in the Orange River (Palmer et al. 2007). Winter flows are the most critical for control, preceding spring outbreaks, when most of the population occurs as larvae/pupae (Rivers-Moore et al. 2007, 2008b). Ideally, larvicide is applied in the low flow months (July), typically using applications of *Bacillus thuringiensis* var. *israelensis* (Bti; Vectobac®). A second organophosphate larvicide (Abate®200EC; active ingredient = Temephos) is also used, and can be applied in higher concentrations and lower volumes, making it an easier larvicide to use under higher flow conditions. In 2001 outbreaks were severe in spite of the control programme, attributed to a mix of extreme high flows, confirmed larval resistance to the more easily applied Abate® organophosphate larvicide (Palmer and Rivers-Moore 2008), and neglect of monitoring and reporting (Palmer et al. 2007). While this programme has generally been effective in controlling blackfly, it has further been shown that applications could be optimized to reduce costs, by making use of downstream carry of the larvicide (Rivers-Moore et al. 2008). Control of blackfly larvae needs to be balanced against the recommendation by Palmer (1997) to leave certain sections of the river untreated as refugia for non-pest blackfly which perform an important ecological function.

Specific new blackfly control issues

During 2011, blackfly outbreaks in the middle and lower reaches of the Orange River were noted to have worsened. It was proposed that current reduced success in controlling *S. chutteri* using larvicides could be one of the reasons for this. Alternatively, or additionally, outbreaks may be due to other species of blackfly not being targeted for control. In the past two years, losses of livestock units have increased due to increased numbers of adult *Simulium* species being observed swarming around and biting livestock. Differences in the behaviour of adult *Simulium* species from the usual behaviour patterns of *S. chutteri* led to speculation by the Northern Cape Agricultural Union that S. *chutteri* alone may not be causing the renewed outbreaks. These differences included reports from local farmers suggesting that the daily window period of exposure to adult *Simulium* larvae has extended (i.e. adult flies are active for periods of the day atypical of behaviour of *S. chutteri*), and that

the morphology of flies differs from *S. chutteri*. The possibility was raised that a secondary species (*S.* (Pomeroyellum) *impukane*), whose larvae breed in slow-flowing streams, was responsible for the increased blackfly problems regionally. Notably, control measures would not be targeting slower-flower river reaches, backwaters and tributaries where this species would be breeding.

The aim of this study was to investigate the hypothesis that the problem is caused by *Simulium chutteri* with recent outbreaks due to a lack of continuity in the control programme, versus the competing hypothesis that the problem is as a result of outbreaks of previously non-problematic species of blackfly, for example *S. impukane*, which have become problematic due to changes in ecological conditions. Answering these questions centres on understanding whether species have different hydraulic and thermal preferences, which would govern generation times (i.e. number of times adult blackfly emerge throughout the year, and how numbers increase) and larval outbreak periods.

Blackfly biology& governing variables

Good descriptions of general blackfly ecology and biology are given in de Moor (2003) and Crosskey (1973). Blackfly are extremely efficient filter-feeders and very well adapted to life in flowing waters. They feed on diatoms, algae, microscopic invertebrates and bacteria. Palmer and O'Keeffe (1995) found that species diversity of blackfly was greatest in mountain headwaters, possibly linked to food limitation in the upper catchments. Blackfly could be classified into "outlet" taxa (non-specialized feeding structures; opportunistic species) versus upper reach taxa (highly specialized fan structures). Rivers-Moore et al.'s (2006) study, building on previous researchers work, again highlights the importance of hydraulic conditions in determining blackfly occurrence. Sheldon and Oswood (1977) developed a food limitation model and verified that increased organic loading (seston) below and impoundment will cause major increases in filter-feeders in the outfall region below a dam. Here, *Simulium* density decreased linearly downstream of an outlet. In summary, the variables governing blackfly larval numbers are velocity, available habitat and water temperature (Rivers-Moore et al. 2008b).

S.*(Metomphalus) *chutteri

Simulium chutteri is one of six southern African species of the bovis group, and prefers swift turbulent waters of large rivers. Palmer (1997, p. 17) lists *S. chutteri* as abundant in large rivers, and a mammalian pest. Larval abundances typically correlate with flow velocities, with very high pest densities typically occurring for flow velocities > 1 ms^{-1}, with an apparent upper limit of 2-3 ms^{-1} (Rivers-Moore et al. 2007).This is largely a result of feeding efficiency and available habitat increasing above these current speeds. In the Great Fish River, a sharp inflection point of available habitat has been identified at 2 m^3s^{-1}, or 2-6 m^3s^{-1}), while Palmer (1997) identified a sharp inflection point in available habitat between 233-603 m^3s^{-1}, and a threshold of *ca*. 300 m^3s^{-1} in the Orange River. Cross-sectional profiles and velocity-discharge curves for the lower/middle Orange River (Palmer 1997) indicates that for velocities of 1 ms^{-1} (*S. chutteri*) discharges would be > 100 m^3s^{-1}.

S.* (Pomeroyellum) *impukane

Simulium impukane is one of seven southern African species of the alcocki group. Palmer (1997, p. 17) lists *S. impukane* as present and widespread in rivers, but not as a pest. *S. impukane* is typically an avian pest, and so the problem is more likely to be one of nuisance value (labour in the grape industry) than mortalities of livestock. Economically, impacts are thus more likely to be due to lost labour days, with potentially chicken deaths in poultry farming areas (for example, Kanoneiland). The behaviour of *S. impukane* adults was noted as being different from *S. chutteri* adults. In the former case, adults go into houses and hotels (Mansfield, 2013, pers. comm.), while in the latter case adults exhibit resting behaviour in shady areas or under plant cover when air temperatures, relative humidity is low and/ or wind speeds exceed 4.1 $m.s^{-1}$ (Myburgh 2003). *S. impukane* larvae generally occur in clear mountain, foothill, and temporary streams, and are tolerant of moderate pollution (de Moor 2002, 2003). Palmer and O'Keeffe (1995) found that *S. impukane* was not associated with dams, and was typical of the upper reaches. Larvae generally attach to dead leaves in slow-flowing waters of small streams, although some species of this group (for example, *S. bovis*) do occur in faster water and larger rivers (de Moor 2002). Rivers-Moore et al. (2006) found that highest densities of *S. impukane* occurred at 0.3 ms^{-1}, having colonised leaves caught on top of stones in current. According to the classification of Davis and Barmuta (1989), *S. impukane* has a preference for hydraulic habitats within subcritical-turbulent flows (Re> 2000; Froude < 1), while *S. chutteri* larvae prefer supercritical-

turbulent flows (Re> 2000; Froude > 1) (de Moor 1994; Rivers-Moore et al. 2007). Both these numbers are dimensionless and describe the mean motion of flow. The Reynolds number indicates whether flows are laminar or turbulent (Re < 500 = laminar and Re > 2000 = turbulent). The Froude number is based on depth, velocity and acceleration due to gravity, and represents the ratio of inertial forces to gravitational forces describes whether flow is sub-critical (Fr < 1; more tranquil flows), critical (Fr = 1) or super-critical (Fr > 1; broken white water) (Davis and Barmuta 1989). Thus, *S. impukane* selects turbulent but slower flowing biotopes, while *S. chutteri* has a preference for turbulent, "white waters". These habitat preferences relate to the feeding efficient and labral fan filtering ability for different species at different flows (Barber-James 2004). For *S. impukane*, given its hydraulic preferences, Rivers-Moore et al. (2006) postulate that small changes in hydraulic conditions caused by small changes in discharges (or even entrapped leaves) may have large impacts on habitat suitability and larval densities. Cross-sectional profiles and velocity-discharge curves for the lower/middle Orange River (Palmer 1997) indicates that for velocities of <0.3 ms^{-1} (*S. impukane*) discharges would be < 2 m^3s^{-1}.

SETTING HYPOTHESES USING STAKEHOLDER INPUTS

A gap analysis was undertaken based on discussions emerging from an expert workshop involving targeted role-players in the blackfly control programme. Key people were invited from the private sector company who supply larvicide for the Orange River blackfly control programme and the National Department of Agriculture (See Appendix I for list of attendees). This meeting was held from 09h00-13h00 on 23 August 2012 at the Philagro[1] SA head office in Pretoria. Meeting objectives and discussion centred on the following points:

- Define current nature of the problem
- Capture experiences in dealing with the current situation
- Define cost of problem
- Define what has changed
- Set hypotheses on the cause of the problem

Discussion points

History of the current problem

More severe outbreaks of blackfly have been occurring periodically over the past ten years, in spite of the current control programme using larvicides. This is corroborated by the monitoring data, based on the 10 point logarithmic scoring scale of Palmer (1994), from 14 sites along the Orange River between August 2009 and August 2012. While monitoring data were missing for 2011, data indicate high scores for 2010 (Figure 1; Kgotla 2012, unpub. data). It was noted that this was not the case between 1992 and 2002 when the control programme was first implemented, based on recommendations by Palmer (1997). Mention had previously been made that occasional outbreaks could be being caused by *S. impukane* (Palmer 1997). Some five years ago, Dr Palmer was approached by the Water Research Commission to investigate this issue further. However, this research was postponed due to the unpredictability of the outbreaks (tentatively linked to *S. impukane*), and because it was difficult to synchronise the WRC funding cycle with blackfly outbreak (biological) cycle.4

[1] www.philagrosa.co.za, 379 Queens Crescent, Sanwood Park, Lynnwood, Pretoria

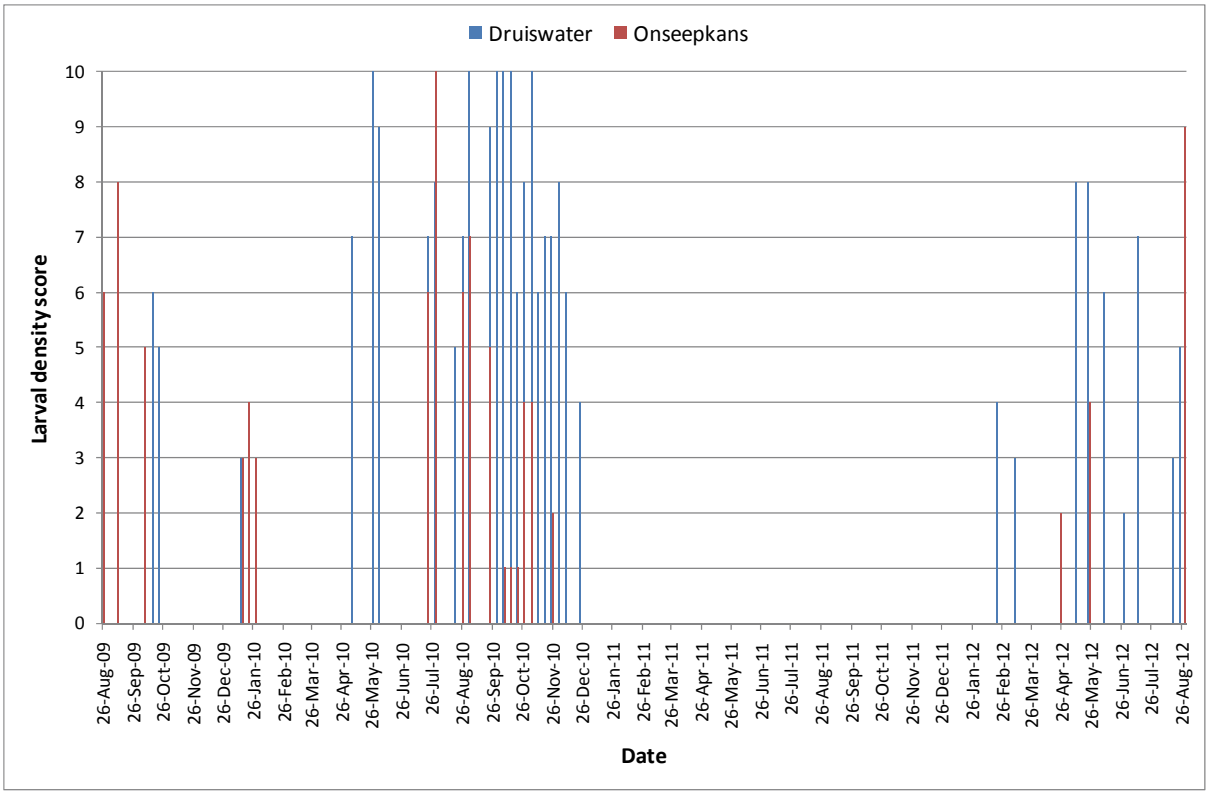


Figure 1 Monitoring data between August 2009 and August 2012 for two monitoring sites on the middle Orange River, and based on the 10-point scoring system of Palmer (1994). Scores above 6 are considered high enough to indicate pestilential outbreak densities (Rivers-Moore et al. 2007).

More recent outbreaks prompted the need for this research to be reconsidered, which resulted in the current research project. For example, at the workshop, it was noted that in spite of recent applications of larvicide, monitoring staff had observed high numbers of larvae on reeds (i.e. slower-flowing side-channels, which could be *S. impukane* larvae) two weeks previously at Blouputs (see Figure 2b for location).

Current Control Programme

The main blackfly problem area is the middle Orange River between Vanderkloof Dam and Onseepkans, with the managed area covering 850 km between Hopetown(river reach 1) and Onseepkans (river reach 8) (Figures 2a,b). Additional details on the monitoring programme may be found in the literature (Palmer 1997; Palmer and Rivers-Moore 2008; Rivers-Moore et al. 2008). The current control programme is based on weekly monitoring and applications of the bacterial larvicide Vectobac®[2]. Logistics for the control programme are divided

[2] Two bacterial larvicides (Teknar® and Vectobac®) and one organophosphate (Abate®) are registered to control blackfly in South Africa

between the Upington and De Aar Agricultural offices, which deal with the Orange River from Hopetown to Sishen Bridge, and Sishen Bridge to Goodhouse/Onseepkans respectively (Figure 1b). The most recent application was the critical winter application (first week of August), starting from Goodhouse and working upstream. It was highlighted that the winter dose is much higher (10 000 litres; concentration = 2.4 ppm) than the summer dose (5000 litres; concentration = 1.6 ppm) to afford more certain control of the crucial winter population. All present at the workshop who were involved with the programme agreed that the approach usually achieves good control.

Previously, applications were done using a Department of Agriculture helicopter with a load capacity of 800ℓ. This has subsequently been put out to tender, and applications are now done by a private contractor, although the currently used helicopter has a smaller load capacity (200ℓ). Volumes of larvicide (dosage) are calculated based on flow volumes, whose accuracy based on data from gauging weirs is an ongoing problem, and downstream carry. Previously, flows were assessed from the most accurate gauging weir (Neusberg – D7H014), and currently flows are assessed using recent data from 3-4 weirs, including Neusberg.

In summary, none of the variables in the control programme has changed over the past ten years, except for the change in larvicides: originally a mixed strategy of the organophosphate Abate® (active ingredient = temephos) and Vectobac® (active ingredient = *Bacillus thuringiensis* var. *israelensis* or *Bti*), and now solely Vectobac®. It was noted that both larvicides have different mixing properties which impacts on downstream carry (Ian Garden, 2012, pers. comm.). *Bti* is more viscous than temephos, and reaches areas with reeds less well than temephos.

Key issues emerging from discussion

Two key points emerged from the workshop discussions, viz.

1. The problems began after resistance to temephos developed and applications only with *Bti*. It was additionally noted that temephos had better penetration into backwaters than *Bti*, and that current applications may not be reaching backwaters.

A relevant question to ask is if Bti is target-specific (blackflies) but not species-specific, why are Bti applications not targeting *S. impukane*.

2. Resulting from the changeover from a National Department of Agriculture (NDA)-owned helicopter to a contractor-owned helicopter (appointed each year through a tender process), no dosage took place during 2010. It was noted that the most recent winter spray was the first comprehensive one "in a while".

Figure 2a Highlighted area showing region of control for blackfly along the middle and lower Orange River (Source: Palmer and Rivers-Moore 2008)

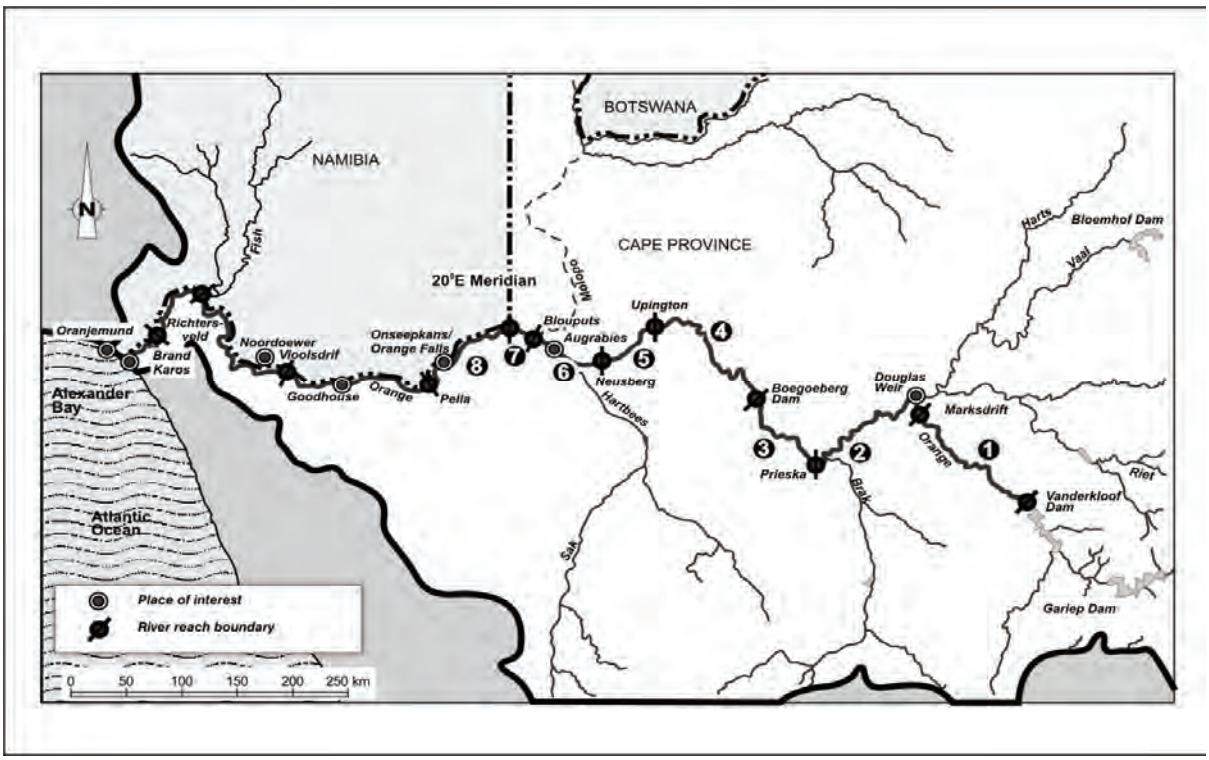


Figure 2b Blackfly control area along the Orange River (reaches 1-8), showing major names and gauging weirs (Source: Rob Palmer)

Thus, two hypotheses emerged from discussions:

- H_1 = The problem is caused by larvicides not reaching the reed habitat, with *S. impukane* larvae not being treated, and which could be resolved through applications of temephos. A critical untested assumption, based on previous research, is that because resistance has been confirmed for Orange River *S. chutteri* through cross-checking with susceptibility of population on the Great Fish River (Palmer and Rivers-Moore 2008), resistance has also developed for *S. impukane*. It is important to test this assumption, which may be false and that resistance may not have developed in *S. impukane* populations due to differences in habitat and the higher likelihood of residual populations of *S. impukane* occurring, which were never exposed to temephos.
- H_2 = The problem is not related to *S. impukane*, and the perceived "worsening of problem" may be anecdotal/ subjective, and is rather contingent on the smooth functioning of the tender award process. This would require investigation of the history of the spraying programme over the past five years, to understand when spraying has occurred and when this programme has not been implemented due to changes in the helicopter contract. This could include correlative assessments with monitoring data where this is available.

Way Forward

Three steps were identified from the workshop, with the intention of addressing the hypotheses outlined above, viz.:

- Undertake trials with Abate®200EC (BASF as registered sole supplier), as residual populations of *S. impukane* are less likely to have built up resistance to temephos as a result of different levels of exposure due to occupation of different habitat. This avenue of research was not pursued as Abate®200EC, while registered for use against blackfly, was not available in South Africa through the registered sole supplier . Importation of this larvicide would only be commercially viable if large quantities were imported, and there would have to be a strong case to do so because of the non-discriminatory action of the key ingredient in aquatic ecosystems, and the potential environmental concerns.
- Obtain spreadsheets of monitoring data from NDA, for time series analyses of when outbreaks occur, and detection of possible patterns.

- Undertake field surveys to confirm presence of *S. impukane*, and categorise likelihood of this species being problematic based on habitat studies (flow velocity, turbidity and water temperatures).

CONFIRMATION OF NEW LEVEL OF OUTBREAKS (THREATS ANALYSIS)

Introduction

The literature review describing hydraulic preferences of *S. chutteri* and *S. impukane* provided a basis for guiding field assessments on the middle Orange River to confirm the presence and habitat preferences of both species of *Simulium*. This therefore required an approach of collecting larval material from a range of habitats covering a range of flow velocities. The objectives of this were to confirm the presence or absence of *S. impukane*, and to relate blackfly species collected in the Orange River to flow velocities. The model of Palmer and Craig (2000) was used as the conceptual framework for the data collection, since the model postulates that the presence of blackfly species can be characterised by a two-dimensional plot of seston preference (turbidity levels) and flow velocity. For this study, a third dimension of water temperature was introduced, since water temperatures are likely to effect the number of generations of blackfly during the year (Rivers-Moore et al. 2012), and different species of aquatic macroinvertebates have been shown to exhibit different levels of sensitivity to thermal thresholds (Dallas and Rivers-Moore 2011). It was therefore reasonable to assume that the different species of blackfly occurring in the Orange River, which have different preferences for hydraulic habitat, may also respond to different water temperature regimes.

Methods

Study area

A total of twelve sites on the middle Orange River between the towns of Upington and Keimoes were surveyed from 27-29 November 2012 (Figure 3). Ideally, sampling would have targeted the lower flow period succeeding winter where larval populations are typically higher (Palmer 1997), but due to the timing of study components which needed to precede the survey, this was not possible. It has also been noted that planning a survey to coincide with *S. impukane* presence is difficult to schedule (Palmer, 2012, pers. comm.). The cluster of sites upstream of Keimoes occurred in a highly anastomosed reach of the Orange River (Figure 4). Here, owing to the underpinning geology, the single channel of the Orange River splits into multiple channels, with flow volumes and flow velocities greatly reduced within each channel. While this site appears to be the largest anastomosed section along the middle

Orange River, such sections occur at intervals along the middle Orange River, coinciding with granitic intrusions within the wider geology of this system. This section is approximately 6 km wide and 9 km long. Upstream and downstream of this, the Orange River reverts to a single channel, except in the vicinity of UP6 (Kanoneiland), where the river divides into two channels with similar flow volumes. Numerous canals diverting water away from the Orange River to surrounding agricultural areas (mostly vineyards) occur, together with channels with slower-flowing agricultural return flows which have high salinity levels. Short site descriptions are provided in Table 1.

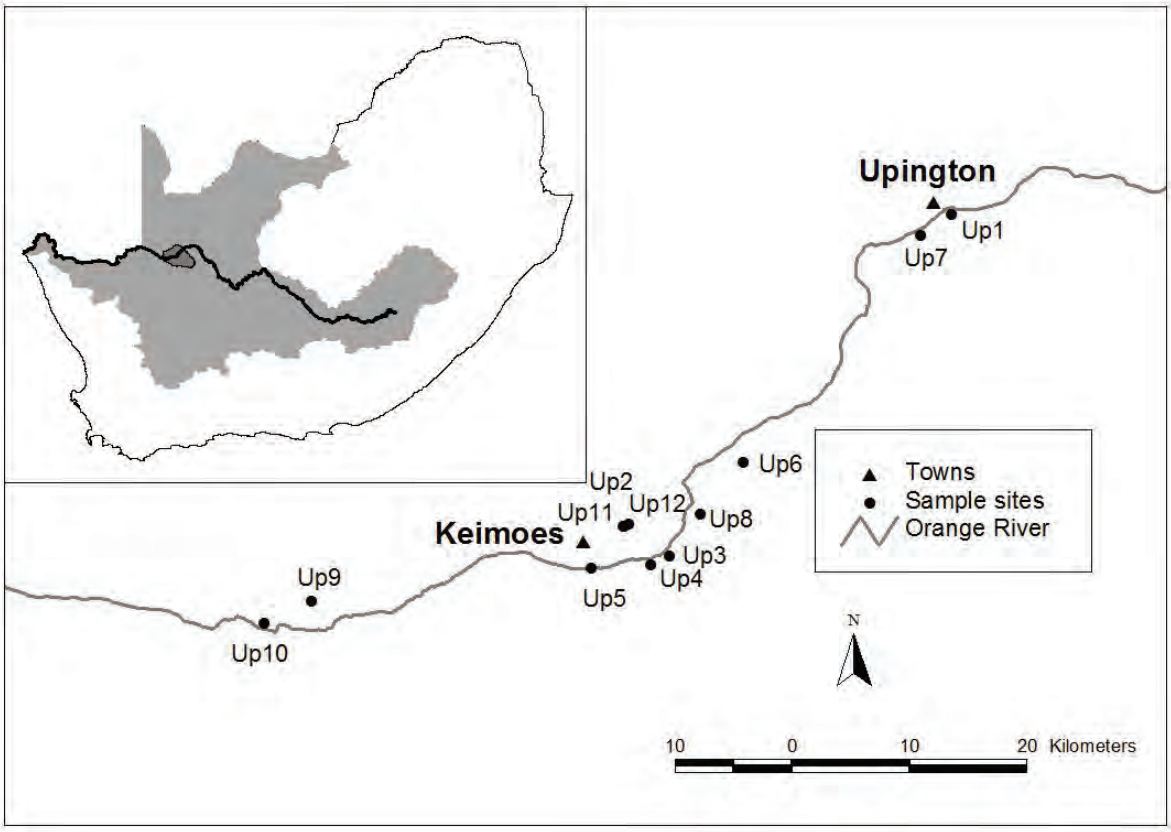


Figure 3 Locations of sites sampled along the middle Orange River between Upington and Keimoes

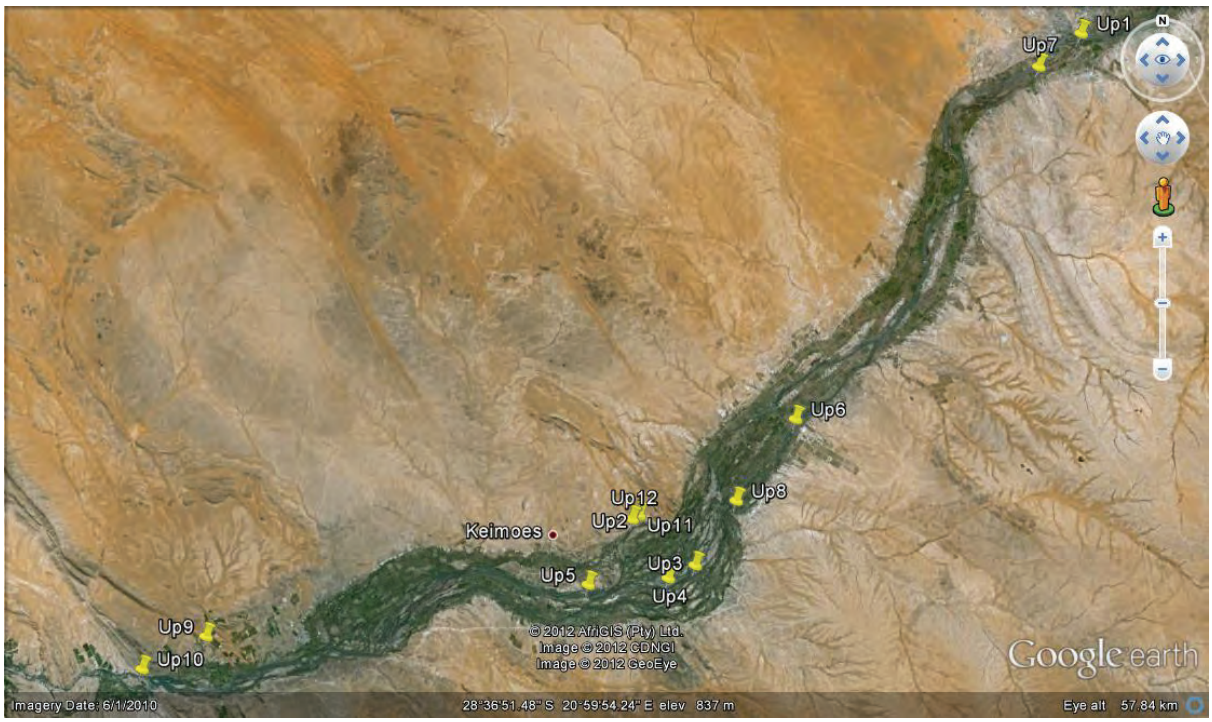


Figure 4 Image of Orange River showing single-channel versus anastomosing sections, and location of sample sites relative to channel types

Table 1 Brief descriptions of sample sites UP1-12

Site	Altitude (m)	Water temps	Notes
UP1	779		Main channel; adjacent to railway bridge near Upington
UP2	675	Y	Side channel
UP3	733	Y	Side channel
UP4	737	Y	Side channel (bedrock-dominated)
UP5	741	Y	Main channel
UP6	734	Y	Main channel (Kanoneiland weir)
UP7	738		Channel with return flows
UP8	711		Main channel and adjacent channel with return flows
UP9	756		Location of poultry farm (Warmsand Pluimvee Plaas)
UP10	773		Neusberg Weir; not able to access river
UP11	766		Side channel
UP12	699		Side channel

Hydrological Assessments

Hydrological analyses were undertaken for flow time series data chosen from eight potential gauging weirs on the middle and lower Orange River (Table 2). Time series data were split into pre- and post-impoundment groups, with 1975 as the cut-off date which was loosely based on when dams were constructed. Differences in flow signatures between each group

were assessed using metrics describing the magnitudes, timing, frequency and duration of different flow events (Richter et al. 1996), and using the Indicators of Hydrological Alteration software (TNC 2006). Further analyses included flow duration curves linked to particular discharges. Threshold discharges were selected using optimal flow velocities for *S. chutteri* of 1 ms^{-1} and 0.3 ms^{-1} for *S. impukane* (Rivers-Moore et al. 2006, 2007), which were related to discharges based on discharge-velocity curves from Palmer (1997).

Table 2 Gauging weirs and record lengths for which flow data were available

Gauging weir	Name	Data Available
D7H002	Prieska	1959-2013
D7H004	Kanoneiland	1971-1989
D7H005	Upington	1936-2013
D7H008	Boegoeberg Dam	1932-2013
D7H014	Neusberg	1993-2013
D8H003	Vioolsdrif (a)	1935-2013
D8H004	Onseepkans	1971-2013
D8H009	Vioolsdrif (b)	1979-2013

Water temperature analyses surveys

Hobo®TidBit v2 water temperature data loggers were installed at five sites to provide insights into differences in diurnal temperature cycles between the faster-flowing single-channel areas (*S. chutteri* habitat) and the anastomosed section (potential *S. impukane* habitat). Temperature data loggers were placed in areas coinciding with habitats where blackfly larvae were collected from. Logging intervals were set to twenty minutes, providing 72 readings per day over an approximately 48 hour period. Longer-term time series of hourly water temperatures were obtained for two sites (Marksdrift and Vioolsdrif) from the Department of Water Affairs (RQS; Mike Silberbauer, Harold van Niekerk). Time periods covered did not coincide with the period of study: Marksdrift = February – August 2010, and Vioolsdrif = June 2003 – August 2005.

Ecological Assessments and Thermal Threshold determination

Eco-hydrological surveys

Stratified surveys in selected hydraulic biotopes (rapids and riffles, plus slow flowing backwaters) at a number of sites on the middle Orange River were conducted to collect

Simulium larvae. Rocks and trailing vegetation in riffle/run biotopes were assessed for larvae. Larvae were classified to species in the field using a hand lens to examine gill branching patterns on associated pupae. For each site where larvae were assessed, measurements on channel width, depth, turbidity and flow velocity were collected.

While the original intention was to also collect *Simulium* adults using sticky traps to collect *Simulium* adults, this was not undertaken as it was decided that the larval phase provided the most suitable information. However, a site previously associated with a poultry farm was visited to ascertain whether there were blackfly associated with chickens. Additionally, the regional State Veterinarian was contact to ascertain whether there had been reports of blackfly problems related to avifauna.

Thermal threshold experiments

Simulium eggs and 6^{th}-7^{th} instar larvae were collected for the purposes of undertaking hatching and upper thermal limit (LT_{50}) experiments, to ascertain whether different species of blackfly have different thermal requirements. As water temperatures and flows are inversely linked, it is hypothesized that *S. chutteri* has lower thermal tolerances than *S. impukane*.

The sampling protocol for eggs differed between *S. chutteri* and non-*S. chutteri* species, in response to their different ovipositing behaviours. *S. chutteri* females oviposit by scattering eggs into the water in pools upstream of riffles. The eggs sink to the benthic sediment. Conversely, all other *Simulium* species generally oviposit onto submerged substrates such as *Phragmites* leaves trailing in flows or partially submerged rocks and stones. Three sites in the vicinity of Upington, where sandbars associated with upstream pools occurred, were sampled for *S. chutteri* eggs. The procedure was to disturb benthic sediment which was sieved through a 100µm net, and examined in the field for eggs. Unfortunately no *S. chutteri* eggs could be found, which may have been as a result of the timing of the sampling effort not coinciding with an egg-laying event by *S. chutteri* females. Eggs for other species of blackfly (primarily *S. damnosum*) were collected from sites within the anastomosed reach of the Orange River downstream of Upington (UP2-3, 8, 11-12) by examining trailing vegetation and rocks where blackfly larvae were present, and collecting leaf segments containing egg clusters. Late instar larvae for *S. chutteri* and non-*S. chutteri* species found in

slower flowing habitats were sampled from trailing vegetation such as leaves and reeds at site UP6. Portions of plant material covered in larvae were retained.

Egg and larval samples were immediately transferred to cooler boxes with ice bricks, and sent by overnight courier to suitable laboratory facilities in Cape Town. These facilities had been prepared to undertake thermal tolerance experiments at a range of temperature intervals according to the approach of Dallas and Ketley (2011) and Ross-Gillespie (2013, pers. comm.). Selection of experimental temperatures was guided by *in situ* temperatures previously recorded for the Orange River in the vicinity of the collection points. Average temperatures were approximately 23^{o}C and experimental temperatures were thus chosen to represent a range above and below this temperature. The highest temperature possible using the aquarium heaters was 37^{o}C.

The aim of this component of the study was to determine the upper thermal limits for *Simulium* species by estimating the thermal thresholds for two life history stages, namely eggs and larvae. This would enable the feasibility of using thermal threshold approaches on *Simulium* species to be examined.

Egg development and hatching

On arrival eggs were assessed with a compound microscope to check which ones were the least developed. Two suitable eggs masses were found attached to pieces of vegetation. These were each divided into six smaller pieces (sub samples) and placed in 10cm diameter petri dishes (~1cm deep) with 50 ml of filtered, de-chlorinated and aerated tap water. Each petri dish held two replicates, where replicate 1 had between 200-450 eggs and replicate 2 between 40-90 eggs. The petri dishes containing the replicate egg samples, barring the dishes at 10^{o}C which were placed on a work bench at ambient air temperature, were floated in small aquaria filled with tap water and heated at different experimental temps (10, 15, 20, 25, 30**,** 33.5^{o}C) using calibrated aquarium heaters. Thermal experiments were undertaken in a controlled temperature environment set at 10^{o}C with full spectrum fluorescent lights on a 12:12 cycle. Small air stones gently aerated the water in the tanks for better heat distribution in the tank. Petri dishes were checked daily for signs of hatching and filled with fresh aerated water.

Date of oviposition was estimated by comparing egg development photos taken of the Simulid eggs obtained from the Orange River (on arrival) to those of the Trichopteran *Chimarra ambulans* (V. Ross-Gillespie, unpublished data) since Trichoptera and Diptera share fairly similar embryonic development). It was estimated that, given an approximate water temperature for the Orange River at the time of collection of ~24-25^{o}C, all eggs had undergone roughly five days of development prior to collection in the field. While these eggs were not unidentifiable to species level, it is assumed they were *S. damnosum* (Palmer 2013, pers. comm.).

Larval rearing

The first newly hatched first instar larvae were photographed using a dissecting microscope with fitted camera and head capsule measurements subsequently recorded of at least 20 individuals from each of the two egg masses. First instar larvae were then separated into replicate vials and placed into the rearing system set at 10, 15, 20, 25, 30, 33.5^{o}C. A known quantity of a mixture of fish flakes and yeast was added to the system for food. Barring the 30 and 33.5°C treatments, newly hatched larvae survived for the first 3 days of the rearing experiment, after which no growth was observed and high mortality was recorded in all treatments. No larvae survived to the stage of pupation or to a stage large enough for positive identification. The rearing experiment was thus terminated.

Larval survival

The LT_{50} (i.e. the temperature at which 50% of the sample survives in a specified time) of *S. chutteri* larvae was determined using the Incipient lethal method (Fry, 1947; Beitinger et al., 2000). This method involves holding duration constant while temperature is varied, with assessments based on survival of a proportion of a sample (Terblanche et al., 2007). Larvae were placed in experimental chambers immersed in glass aquarium tanks, filled with de-chlorinated and aerated tap water, which was heated using aquarium heaters to five different temperatures. Aquarium tanks were aerated using two air stones per tank to ensure that the percentage saturation of dissolved oxygen remained above 75%. This also served to simulate flow. Water temperature was recorded at 15 min intervals throughout the experiment using Hobo TidbiT v2 loggers (Onset Computer Corporation, 2008) placed in each of the tanks. The mean (± standard deviation) experimental temperatures in each tank were calculated as

16.2±0.1, 23.3±0.6, 26±0.1, 30.0±0.2 and 37.0±0.2^{o}C. Twenty three individuals were placed in each experimental chamber and chambers were checked for survival every 24 hours for four days (96 h). No LT_{50} experiments could be undertaken on *S. impukane* as larvae were not present at sites in the Orange River. A widespread blackfly species from the same tribe as *S. chutteri* (Metomphalus), *S. vorax*, was collected as a clear water equivalent to *S. impukane* to determine LT_{50} values for a species with preferences for low seston concentrations (<10 mg/L – Palmer and Craig 2000), even though this species tends to prefer higher velocities (> 1.9 ms^{-1} – Palmer and Craig 2000).

Water Quality analyses

Water quality data were assessed at eight sites, primarily relating to the sites where blackfly were sampled from, but also including sites upstream and downstream of these. Water quality data were accessed from the Department of Water Affairs (DWA) Water Resources Quality website (http://www.dwaf.gov.za/iwqs/wms/data/WMS_pri_map.asp). The water Quality parameters that were identified as important for this study involved Electric conductivity (EC), Nitrate/nitrite and Phosphorous. Nitrogen to phosphorous ratios were calculated, and compared against a threshold based on the Redfield ratio of N:P = 16:1 (Stelzer and Lamberti 2001), where values < 16:1 indicate a nitrogen-limiting environment and ratios > 16:1 indicate a phosphorous limiting environment. Time periods where data were available were not directly compatible at all sites, and data ranged from time series of different lengths to single values. Consequently, only median values have been used. The water quality data from Karos Settlement station 186792 near D7H006 was assumed to represent water quality before it reaches and passes through the Upington area. It is important to note that 3.2 km downstream of site UP7, there is a waste water treatments works .

RESULTS

Hydrological Assessments

Hydrological assessments showed distinct changes in pre- and post-impoundment flows at all gauging weirs assessed. Data are presented below for the flows at D7H005 (Upington), with the results from additional analyses presented in Appendix II. The pre-impact period was defined as falling between 1943 and 1975 and the post period was between 1976 and 2012. The mean annual stream flow recorded decreased from 359 m^3s^{-1} to 218.9 m^3s^{-1} for pre- versus post-impoundment periods. Mean monthly flows for the pre-impoundment period between October and May were lower than for post-impoundment mean monthly flows. However for June to September flows for the post-impoundment period were similar or greater than the pre-impoundment period (Figure 5). The mean annual coefficient of variation increased from 1.64 for pre-impact to 1.93 for post impact (Table 3). Changes on monthly flow patterns were particularly pronounced when flow volumes for the preceding two years were compared with mean monthly flow volumes for a sixty year period (Figure 6). Impacts on 7-day maximum and 7-day minimum flows showed inverse patterns. Post-impoundment flows exhibited a decrease in the maximum 7 day flows compared to the pre-impoundment flows (Figure 7). Conversely, the 7-day minimum stream flows increased in the post-impoundment period compared to the pre-impoundment period (Figure 8). In both situations, flows remained within a single standard deviation of the mean, although mean values have decreased for the 7-D maximum flows and increased for the 7-D minimum flows. Analyses using flow duration curves showed that flows in the main channel are generally favourable for *S. chutteri*, but do not drop to levels favourable for *S. impukane* (Table 4 and Figure 9)

Table 3 Statistics for the five Indicators of Hydrologic Alteration flow group types defined by Richter et al. (1996) for D7H005. Pre- and post-impact periods were defined as 1936-1975 (39 years) and 1976-2012 (37 years) respectively

	MEANS		COEFF. of VAR.		DEVIATION FACTOR		DEV. of C.V.	
	Pre	Post	Pre	Post	Magnitude	%	Magnitude	%
Parameter Group #1								
October	238.2	121.9	2.268	0.8782	-116.3	-48.83	-1.39	-61.28
November	356.1	170.7	1.141	0.8771	-185.4	-52.06	-0.2638	-23.12
December	468.3	212.5	1.144	1.185	-255.8	-54.62	0.04083	3.568
January	425.6	281.8	1.272	1.643	-143.7	-33.77	0.3703	29.11
February	732.1	432.7	1.131	1.416	-299.4	-40.89	0.2847	25.18
March	703.7	440.4	1.031	1.681	-263.3	-37.42	0.6501	63.03
April	551	287.8	0.8939	1.171	-263.3	-47.77	0.2775	31.04
May	341.1	191	1.158	1.127	-150.1	-44.01	-0.03089	-2.669
June	185.7	167.3	1.148	1.339	-18.35	-9.883	0.1913	16.67
July	96.99	115	0.9941	0.6882	18	18.56	-0.3059	-30.77
August	91.75	98.45	1.708	0.7449	6.696	7.298	-0.9629	-56.38
September	144.3	119.8	1.931	1.135	-24.49	-16.97	-0.7956	-41.21
Mean |%| change						34.3		32
Parameter Group #2								
1-day minimum	14.47	36.76	1.003	0.4309	22.29	154	-0.5721	-57.04
3-day minimum	15.95	38.48	1.099	0.4274	22.53	141.2	-0.672	-61.13
7-day minimum	18.26	42.2	1.18	0.4599	23.94	131.1	-0.7205	-61.04
30-day minimum	29.29	55.2	1.591	0.5251	25.91	88.43	-1.066	-67
90-day minimum	66.54	80.55	1.187	0.6331	14.01	21.05	-0.5535	-46.64
1-day maximum	2526	1268	0.4417	1.246	-1258	-49.81	0.8045	182.1
3-day maximum	2335	1219	0.4581	1.246	-1116	-47.79	0.7878	172
7-day maximum	2038	1113	0.5067	1.247	-925.1	-45.4	0.7408	146.2
30-day maximum	1415	767.1	0.6314	1.304	-647.7	-45.78	0.6726	106.5
90-day maximum	869.1	477.6	0.7107	1.158	-391.6	-45.05	0.4473	62.93
Number of zero days	1.364	3.784	4.361	4.371	2.42	177.5	0.01028	0.2358
Base flow index	0.05303	0.307	0.9827	0.6101	0.2539	478.8	-0.3726	-37.92
Mean |%| change						118.8		83.4
Parameter Group #3								
Date of minimum	245.9	240.1	0.2247	0.1802	5.771	3.153	-0.04449	-19.8
Date of maximum	61.33	49.95	0.2252	0.2143	11.39	6.223	-0.01088	-4.833
Mean |%| change						4.7		12.3
Parameter Group #4								
Low pulse count	4.667	1.865	0.8959	1.475	-2.802	-60.04	0.579	64.62
Low pulse duration	25.13	12.32	0.819	2.619	-12.81	-50.98	1.8	219.8
High pulse count	4.152	1	0.6736	1.633	-3.152	-75.91	0.9594	142.4
High pulse duration	11.9	13.32	1.254	0.5707	1.42	11.93	-0.6837	-54.51
Low Pulse Threshold	36.11							
High Pulse Threshold	946.3							
Mean |%| change						49.7		120.3
Parameter Group #5								
Rise rate	64.67	28.25	0.4462	0.8629	-36.41	-56.31	0.4167	93.39
Fall rate	-39.24	-21.14	-0.4288	-0.8331	18.09	-46.11	-0.4043	94.28
Number of reversals	93.55	121.7	0.178	0.1845	28.18	30.13	0.006483	3.642
Mean |%| change						44.2		63.8

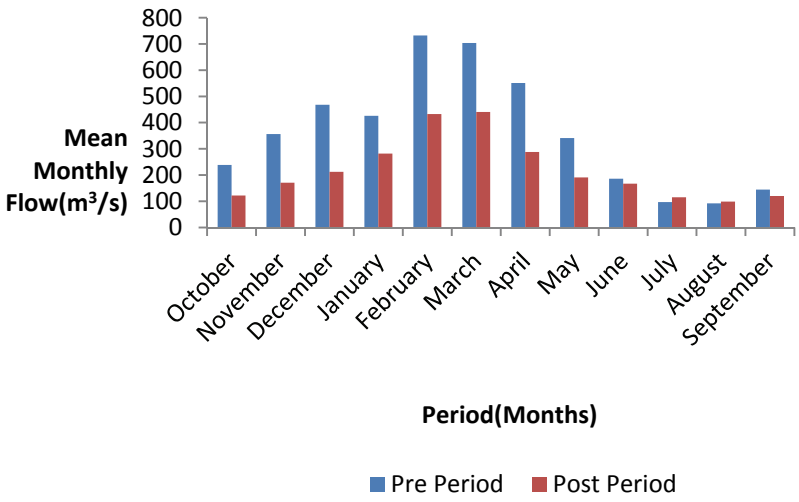


Figure 5 Bar graph showing the mean monthly flow flows for the pre- and post-impoundment period for the Upington weir.

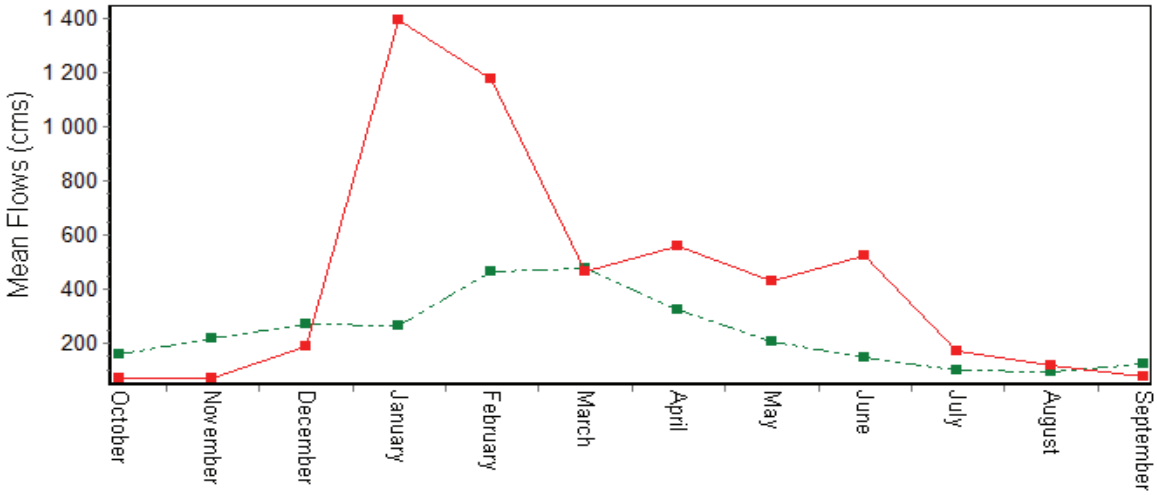


Figure 6 Mean Monthly flows for 1936 -2010 (green) versus most recent (2011 -2012; red line) at Upington.

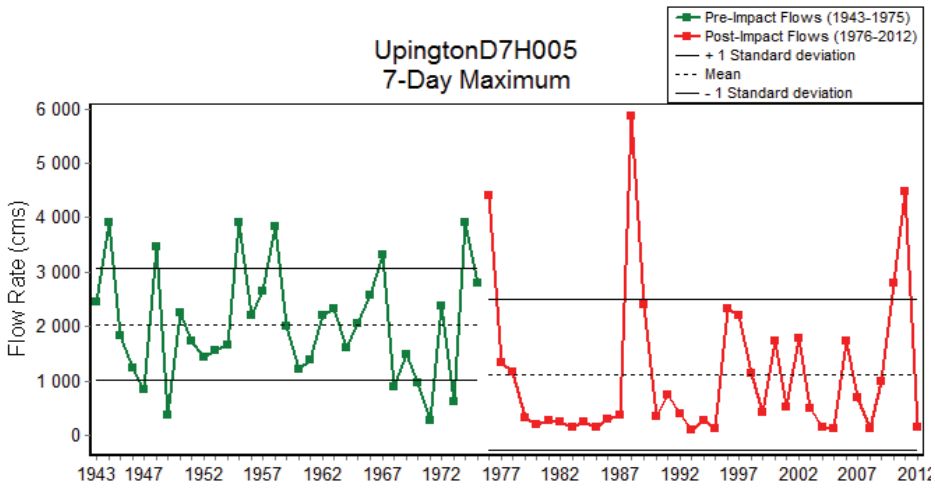


Figure 7 Graph showing the 7–Day Maximum flows for the pre- and post-impoundment periods at the Upington weir D7H005.

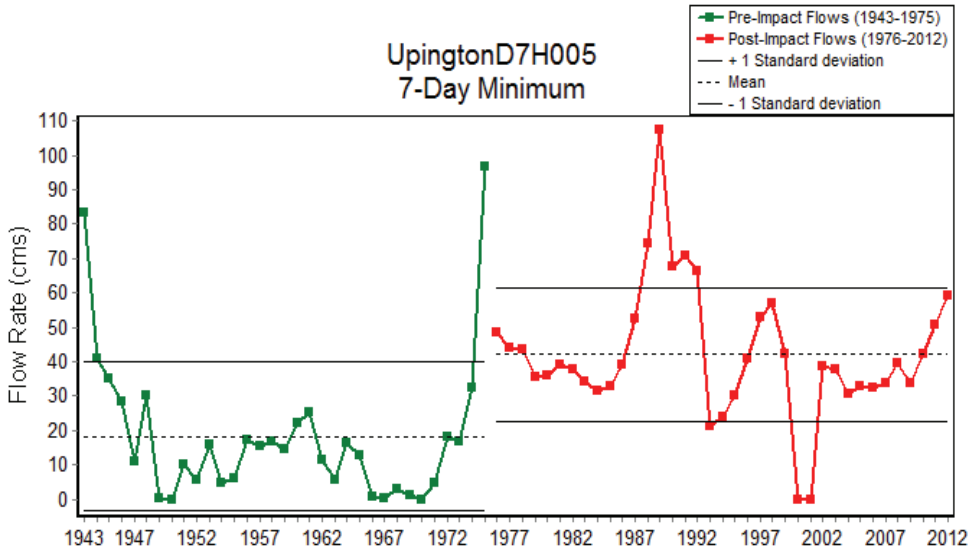


Figure 8 Graph showing the 7-Day Minimum flows for the pre- and post-impoundment periods at the Upington weir D7H005.

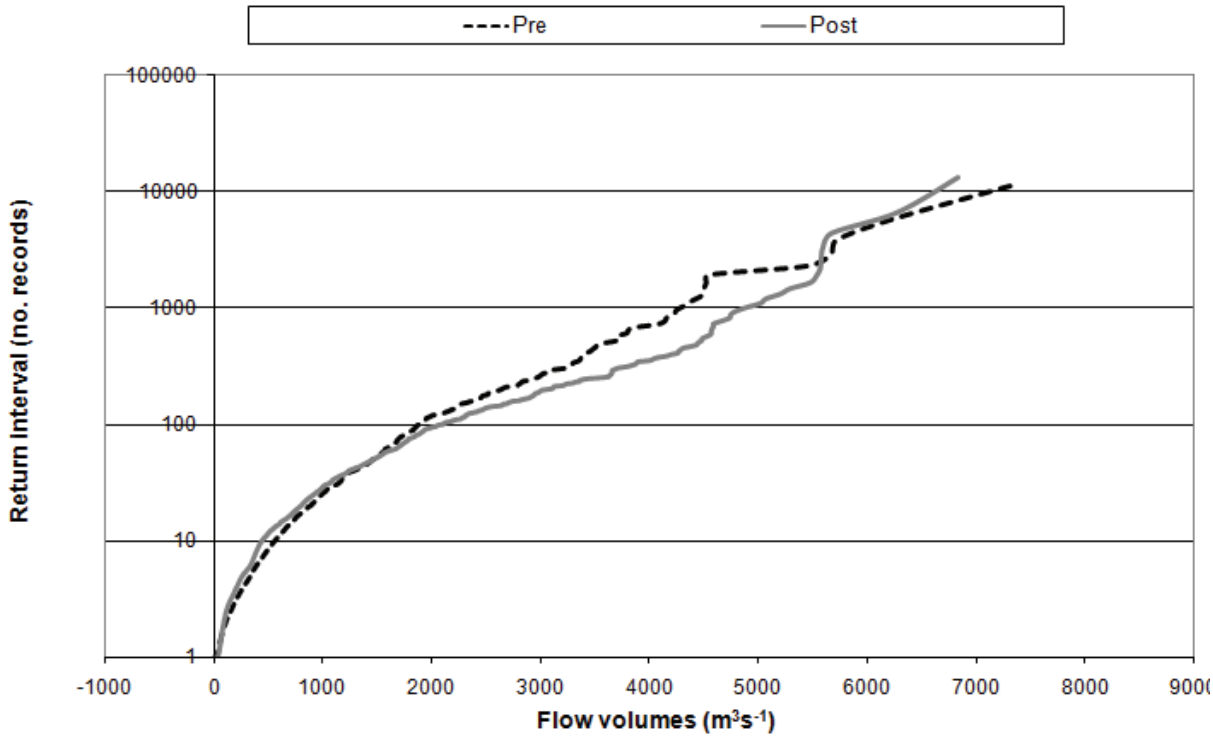


Figure 9 Return interval curves for D7H005 Upington for pre-impoundment (19xx-1975) and post-impoundment (1976-2012) flows

Table 4 Probabilities (%) of flow volumes falling below threshold values relevant to blackfly habitat for Upington (D7H005)

	Flow volume threshold			
	2	**30**	**65**	**100**
Pre	0.99	8.26	35.06	48.98
Post	0.00	0.99	31.03	54.55

Water temperature analyses

Mean water temperatures were similar between sites. However, what was noticeable was that temperature patterns fell into two distinct patterns, viz. pronounced sinusoidal diurnal cycles (UP2, UP3, UP4) versus less pronounced daily variation (UP 3 and UP6) (Figure 10; Table 5).

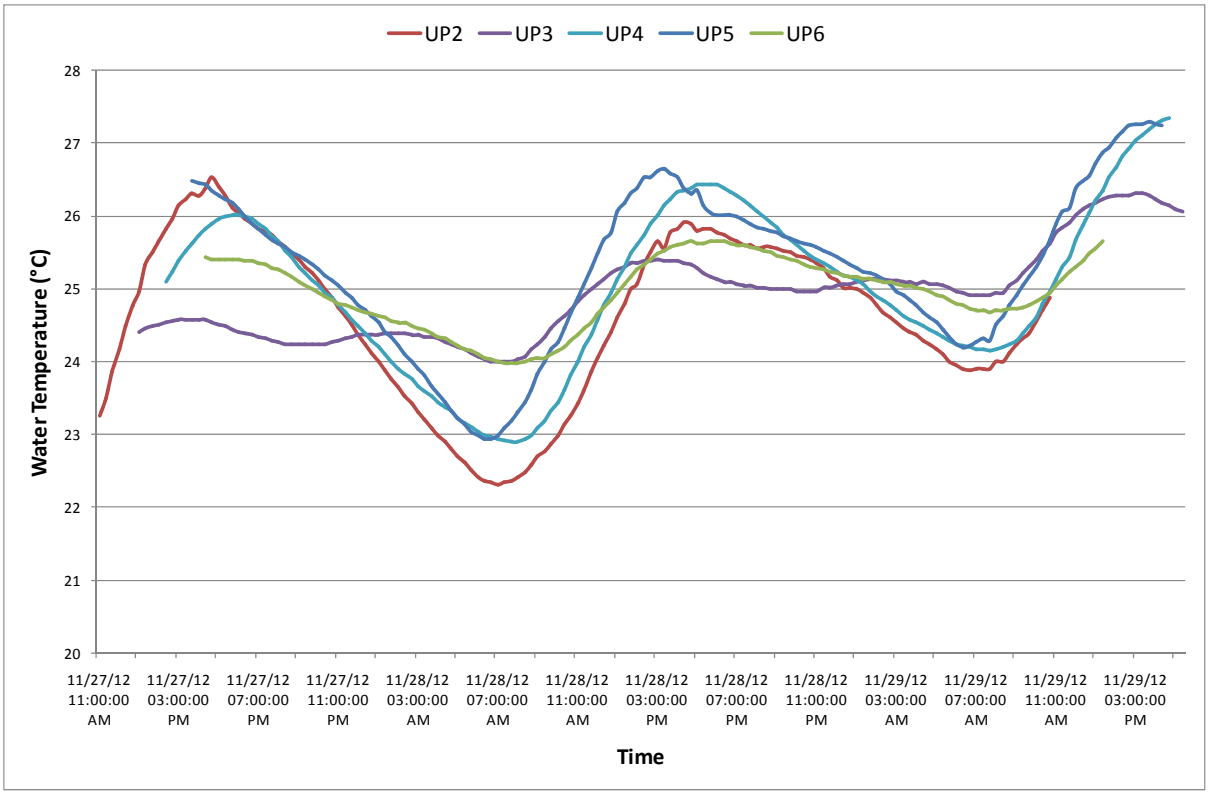


Figure 10 Water temperatures at 20-minute intervals over a 48 hour period from 27-29 November 2012 at five sites from the main channel and anastomosing sections of the middle Orange River

Table 5 Descriptive statistics for water temperatures at five sites along the middle Orange River over a 48 hour period from 27-29 November 2012

	UP2	**UP3**	**UP4**	**UP5**	**UP6**
Mean	24.59	24.92	25.02	25.27	24.95
SD	1.13	0.62	1.12	1.10	0.49
Max	26.52	26.30	27.33	27.28	25.65
Min	22.32	24.00	22.90	22.94	23.98
Range	4.20	2.30	4.44	4.34	1.67

Longer term water temperatures at the upper reach of the blackfly problem (Reach 1 – Marksdrift) and the lowermost reach (Reach 8 – Vioolsdrif) both exhibited seasonal differences in water temperatures (Figures 11-12). Interestingly, both sites exhibited small daily temperature ranges (1.54 ± 0.55 and 1.59 ± 0.93°C for Marksdrift and Viooldrif respectively), with maximum daily ranges only being 3.17 and 4.50°C at Marksdrift and Vioolsdrif respectively. The second year's data from Vioolsdrif was not used, as it appeared

that the recording equipment was faulty vis-à-vis values being in whole numbers with no sub-degree resolution.

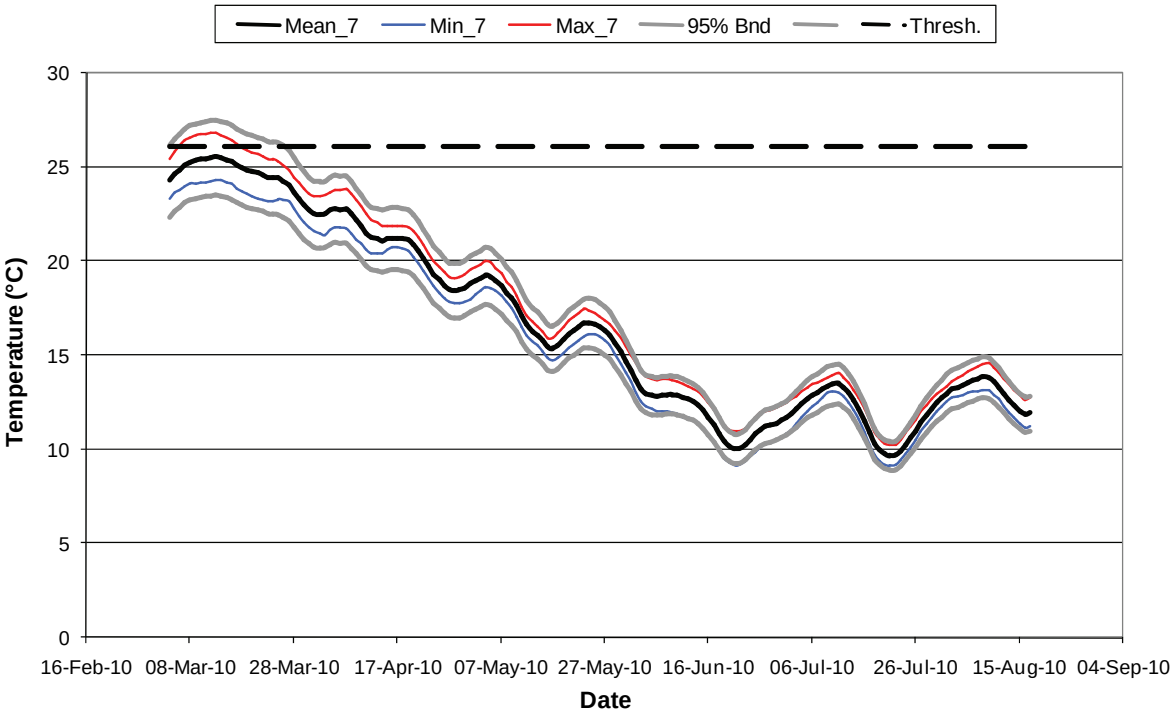


Figure 11 Daily water temperatures (mean, minimum and maximum) smoothed using a 7-day moving average, plus 95% confidence envelopes, for Marksdrift between 26 February 2010 and 17 August 2010. Exceedance of the 26.1°C 24-h LT_{50} threshold for *S. chutteri* (see subsequent sections) is shown.

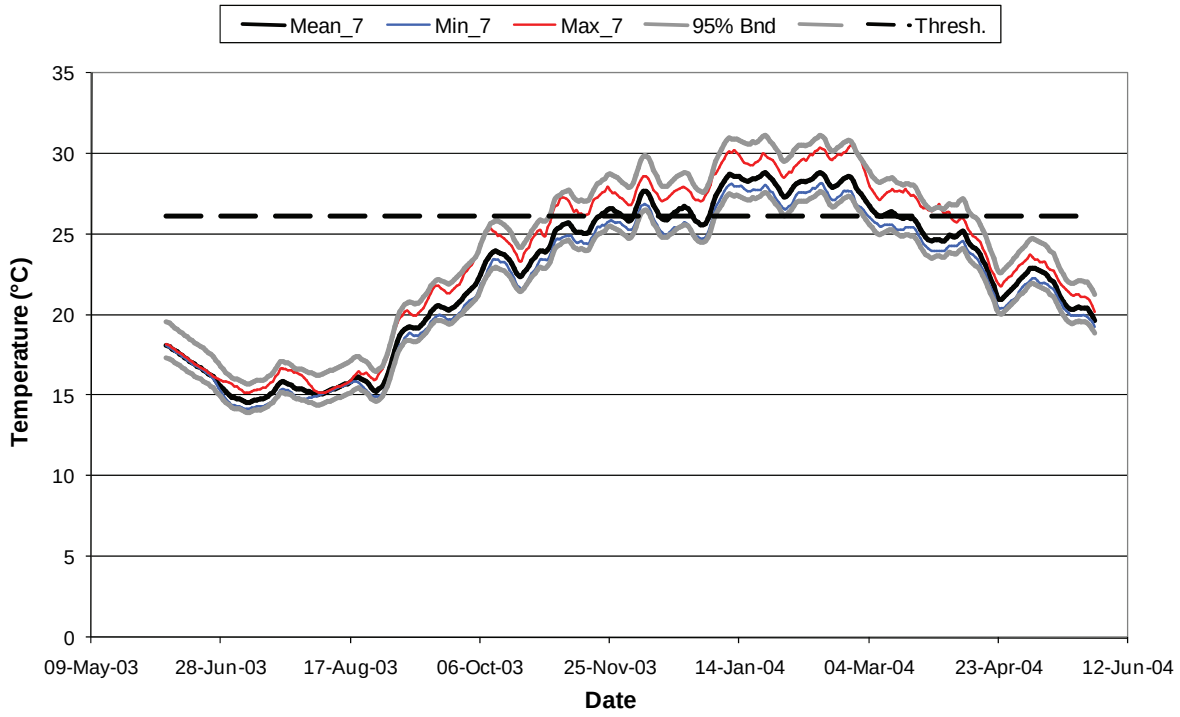


Figure 12 Daily water temperatures (mean, minimum and maximum) smoothed using a 7-day moving average, plus 95% confidence envelopes, for Vioolsdrif between 1 June 2003 and 31 May 2004. Exceedance of the 26.1°C 24-h LT_{50} threshold for *S. chutteri* (see subsequent sections) is shown.

Ecological Assessments and determination of thermal thresholds

Eco-hydrological preferences

Irrespective of site location, turbidity was consistent between the single channel and anastomosed sections of the middle Orange River. The channels with agricultural return flows (UP7 and UP8) had very low turbidity levels (Table 6). Widths, depths and velocities are only reflected for the smaller channels surveyed from the anastomosed sections. Flow velocities were generally below 1 ms^{-1}.

Table 6 Physical characteristics relevant to blackfly habitat from sites sampled in smaller channels

Site	Turbidity (m)	Width (m)	Depth (m)	Velocity (ms^{-1})
UP2	0.24	7.50	0.25	0.50
UP3		5.00	0.70	0.30
UP4	0.24	4.00	0.17	0.50
UP7	>1	1.00	0.15	<0.35
UP8		5.00		0.80
UP11	0.23	2.00	0.27	0.35
UP12	0.23	8.00	0.30	1.20

All sites generally had lower densities of blackfly larvae and pupae than expected, but many rocks were covered in algae and turbidity levels were lower than expected. A total of six species of *Simulium* were recorded, with no individuals of *S. impukane* found (Table 7). There was no means to ascertain whether *S. impukane* adults, which favour avian hosts for a blood meal, were prevalent because the poultry farm was not in business, although a resident in the area who kept chickens had not experienced problems. No problems relating to blackfly on poultry had been reported to the State Veterinarian either, although it was noted that since there was only one remaining poultry farm in the area, chances of such reporting were low. It was further noted that 2011 had been an especially bad year for blackfly outbreaks, with improvements in 2012 (Nel, 2013, pers. comm.). *S. chutteri* was the most abundant and widespread species, while *S. ruficorne* was restricted to the slow-flowing, highly saline agricultural return flow channels. Notably, this was the first time that *S. medusaeforme* has been recorded from the Orange River (Palmer, 2012, pers. comm.). The blackfly species sampled showed distinct flow velocity preferences, with *S. chutteri* being the only species preferring flows > 1 ms^{-1}, while the remaining species showed preferences for lower flows across a spectrum from 0.35-0.68 ms^{-1} (Table 8).

Table 7 Blackfly species occurring at the sites surveyed, with abundances reflected as logarithmic codes (1; A = 2-10; B = 10-100; C = >100) (Sade = *S. adersi*; Schu = *S. chutteri*; Sdam = *S. damnosum*; Smac = *S. mcmahoni*; Smed = *S. medusaeforme*; Sruf = *S. ruficorne*)

Site	Sade	Schu	Sdam	Smac	Smed	Sruf
UP1		C				
UP2	A		B	1		
UP3		B	B	A		
UP4		C		A		
UP5						
UP6		C				
UP7						A
UP8	A		A	A		A
UP11	A	1	B	A	B	
UP12	A	A	B	A	B	

Table 8 Flow velocities (in ms^{-1}) by blackfly species, measured using a flow meter adjacent to where species were sampled

	Sade	**Schu**	**Sdam**	**Smac**	**Smed**	**Sruf**
UP1		1.60				
UP2	0.50		0.50	0.50		
UP3		1.60	0.75	0.30		
UP4		0.90		0.40		
UP6		1.60				
UP7						0.35
UP8	0.95		0.75	0.75		0.35
UP11	0.35	0.35	0.35	0.35	0.35	
UP12	0.90	1.60	0.90	0.90	0.90	
Mean	0.68	1.28	0.65	0.53	0.63	0.35

Thermal thresholds

Egg development and hatching

Trends in egg hatch parameters of *S. damnosum* were plotted using box plots (Figure 13). The degree days have been calculated assuming a lower thermal limit of 0 (as a lower thermal limit for development is unknown), a constant *in situ* water temperature of 25^{o}C (5 days with diel fluctuations not taken into account) and a temperature of 15^{o}C during transportation to the laboratory (1 day). Kruskal-Wallis tests were performed to detect differences in egg hatch parameters among treatments. While Kruskal-Wallis tests were not significant (most likely due to having too few replicates at each temperature), trends are however definitely evident among treatments. Percentage hatch decreases at temperatures of 30^{o}C and above (Figure 13) suggesting that a critical limit for development lies between 30 and 33.5^{o}C, while the optimum temperature for development (based on degree days to mean hatch and the percentage hatch) would appear to be around 25^{o}C. Generally the degree days required to mean hatch in warm adapted species decreases at higher temperatures until a thermal optimum is reached after which it starts increasing again - this is observed in Figure 13. Days to first hatch varied from 13 days at 10^{o}C to 9 days at 25^{o}C. The length of hatch indicates a highly synchronous hatch (on average between 3 and 5 days) at a wide range of temperatures (Figure 13).

A thermal reaction norm based on the degree days to mean hatch (Figure 14) was calculated for this species based on the methods presented by Pritchard et al. (1996). This is useful method for characterising a species egg development and for comparing it against species

globally. Essentially it involves taking the natural log of temperature treatment as well as the natural log of degree day requirement to mean hatch and then performing a regression analysis between these two variables to obtain the slope of the relationship. The slope gives an indication as to whether the species is warm or cold adapted or a generalist species. In calculating the thermal reaction norm temperatures above the optimum (those at which the degree days to mean hatch start an opposite trend relative to the previous points) are excluded. In this case the temperature treatments at 30 and 33.5 were therefore excluded, as at both of these treatments the degree day requirements were shown to increase, this in contrast to the trend calculated for the preceding points.

The thermal reaction norm is -0.05. This values is less than what has been recorded (-2.12 to -0.4) for other Diptera (including members of the Tipulidae, Culicidae and Sciomyzidae) (Appendix 2 in Pritchard et al., 1996) and is in fact closer to reaction norms calculated for some warm-adapted stoneflies (Appendix 2 in Pritchard et al., 1996). A negative slope indicates warm water adaptation, however as this slope is not steep and fairly close to zero this could indicate adaptation to more variable conditions or a shift from warm water adaption to being able to handle cooler waters.

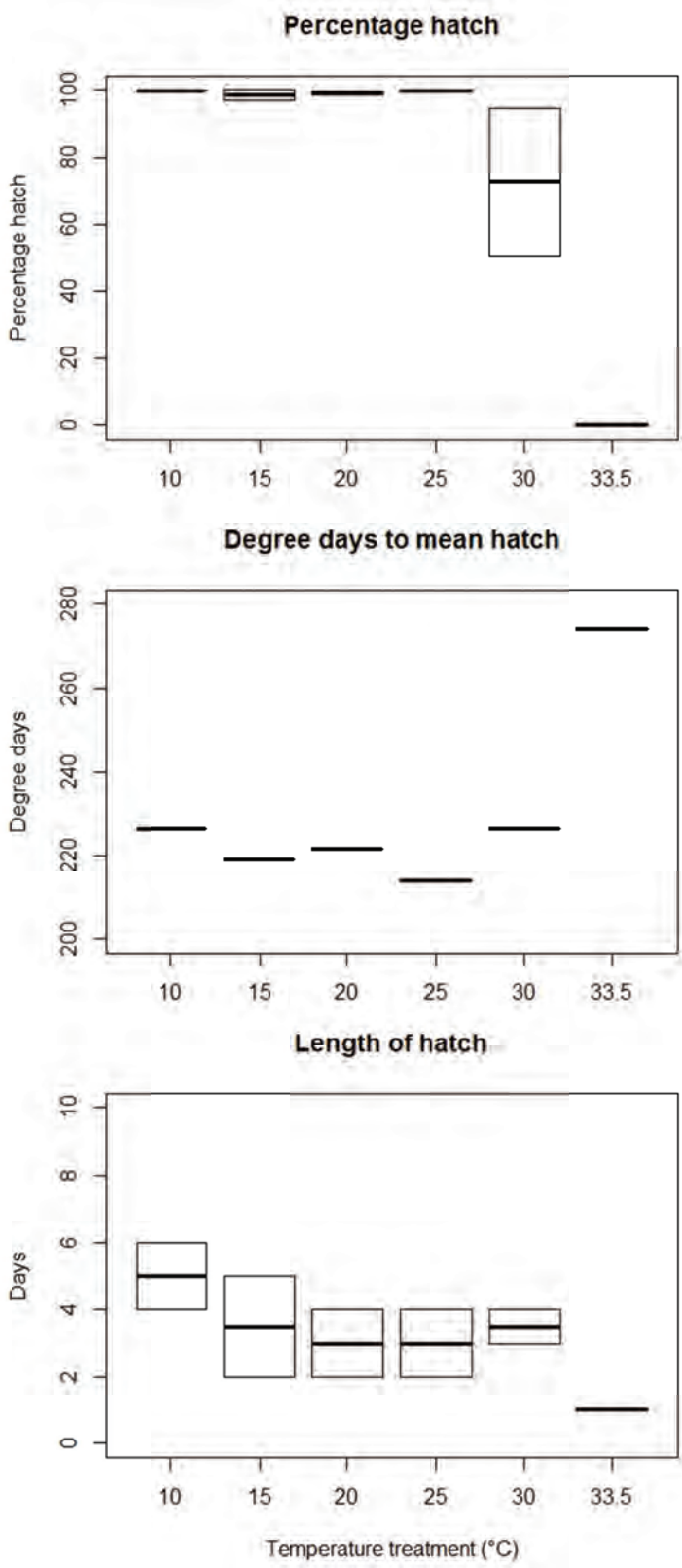
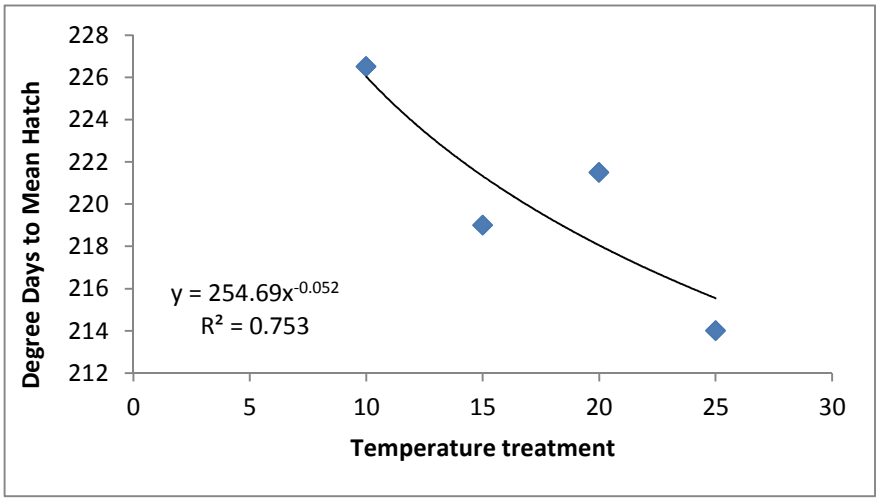


Figure 13 Percentage hatch, degree days to mean hatch, and length of hatch of *Simulium damnosum* eggs at six temperatures (Note: A single line indicates no variance or difference between the replicates)



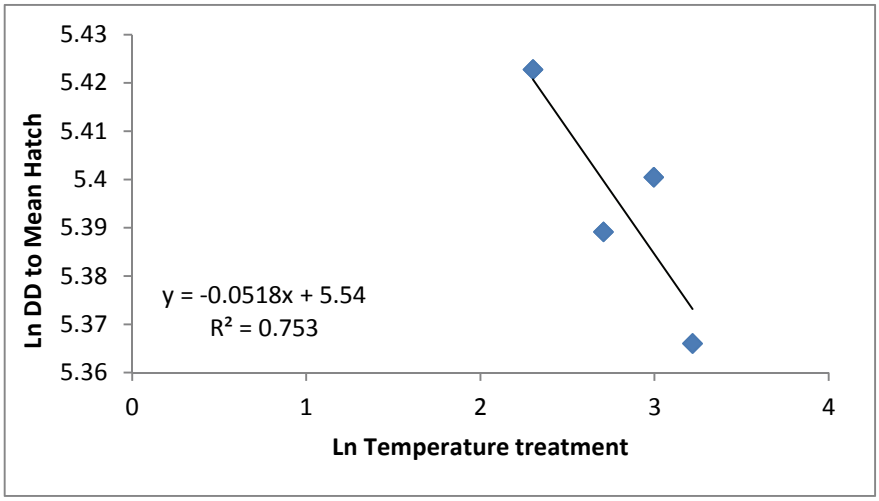


Figure 14 Thermal reaction norms (log-transformed relationship between degree day requirement and mean hatch) at different temperature treatments (°C) calculated for *S. damnosum*. Untransformed power equations defining the same relationship are given for each species.

Larval LT_{50}

It was not possible to determine an LT_{50} for *S. vorax* due to larval mortalities. Likely reasons for this include: experimental temperatures being too high; organisms needing to be identified as *S. vorax* was not the only species present; and acclimation of organisms for 48 hours preceding the experiments. LT_{50} values and 95% confidence intervals for *S. chutteri* larvae were calculated using the Trimmed Spearman–Karber analysis (USEPA TSK Programme Version 1.5). This method is used extensively for estimating median lethal concentrations in toxicity bioassays and has been shown to be accurate, precise and robust and is easily computable (Hamilton et al., 1977). Larval mortalities were observed after 24 hours at all experimental temperatures (Table 9), with 100% mortality after 48 hours at

26.1°C, 30.0°C and 37.0°C. Normally LT_{50}s are calculated for each time period (every 24 h) and plotted against treatment temperature and analysed using linear regression. The resulting regression formula is then used to determine the incipient lethal upper temperature (ILUT). However, given the relatively high mortality rate, it was only possible to estimate the 24 h LT_{50}, which was 26.1°C, with a lower 95% Confidence Limit of 25.1°C and an upper 95% Confidence Limit of 27.0°C. It is likely that the stress associated with transportation of larvae from the point of collection on the Orange River to the experimental facility in Cape Town led to the high mortality rate. The calculated LT_{50} does however provide a first estimate of the upper thermal limit for *S. chutteri* larvae, although it is recommended that additional thermal experiments be undertaken nearer the point of collection to eliminate uncertainty associated with transportation of individuals. Both the short-term temperature data collected during this study, and the longer-term historical data assessed indicate periods of exceedance of this 24-h LT_{50} threshold.

Table 9 Cumulative mortality of *S. chutteri* larvae during thermal experiments

Temperature (Mean ± standard deviation) in °C	**n**	**24-h**	**48-h**	**72-h**	**96-h**
16.2 ± 0.1	23	7	13	18	21
23.3 ± 0.6	23	9	21	23	-
26.1 ± 0.1	23	15	23	-	-
30.0 ± 0.2	23	23	-	-	-
37.0 ± 0.2	23	23	-	-	-

Water quality analyses

Conductivity was relatively constant with downstream distance, with all values exceeding 20.7 mSm^{-1}, and data showing a slight trend of increasing with downstream distance (Table 10). Based on the ratio of N:P, values indicated a nitrogen-limiting system, but with high variability between sites and no downstream trends.

Table 10 Key water quality (conductivity, nitrates and phosphates) median values for eight sites along the middle Orange River, with particular focus on sites in the vicinity of the field survey sites

Site	Survey Sites	EC (mS/m)	$NO_2^- + NO_3^-$ (mg/L)	PO_4^{3-} (mg/L)	N:P
Karos Settlement (D7H006)	Upstream	20.70	0.352	0.023	15.3:1
DWA Station 184055	UP1	32.00	-	0.005	-
Upington (D7H005)	UP7	32.20	0.703	0.056	12.6:1
Kanoneiland (D7H004)	UP2-6, 8, 11	26.20	0.190	0.014	13.6:1
DWA Station 186793	UP2-6, 8, 11	21.10	0.277	0.032	8.7:1
D7H014/D7H016	UP9-10	35.35	0.320	0.025	12.8:1
Onseepkans (D8H004)	Downstream	31.00	0.047	0.019	2.5:1
Goodhouse (D8H005)	Downstream	26.10	0.020	0.003	6.7:1

Synthesis

Field survey

During this survey, larval numbers were not as high as had been expected, although based on the occurrence of multiple species of blackfly larvae at most sites, these low numbers were probably not residual populations remaining after a recent larvicide spraying application. However, what was apparent was that the water was relatively clear (0.23-24 cm secchi depth, versus 9cm on the Great Fish River which typically favours *S. chutteri*; Rivers-Moore et al. 2007), with much of the substrate that would be available to blackfly larvae covered in algae. There is typically a correlation between clear water, low flows and higher levels of turbidity for higher flows, which was what was encountered on this survey.

In spite of not collecting *S. impukane* at any of the sites, six species of blackfly were found across a range of hydraulic biotopes and habitats. This included *S. chutteri* as the overwhelmingly dominant species in the fast flowing main channel habitats, *S. ruficorne* in the slow-flowing clear agricultural return flow channels, and a mix of species largely dominated by *S. damnosum* and *S. mcmahoni* in the slower flowing anastomising sections. What was of particular interest was finding *S. medusaeforme* at two of the sites, since this species has not previously been recorded on the Orange River (Palmer 2012, pers. comm.) The presence of *S. medusaeforme*, a "small water" species, may be as a consequence of faunal changes which have occurred resulting from the Lesotho Highlands water scheme. This species is common in lower foothill streams, together with *S. damnosum* and *S. adersi*

(Palmer and O'Keeffe 1995; Rivers-Moore et al. 2006). The most extensive previous surveys had pre-dated this scheme's completion (Palmer 1997). *S. ruficorne* is often associated with temporary and polluted streams (Palmer and O'Keeffe 1995) has probably expanded its range as a consequence of the channels containing agricultural return flows, which have similar habitat to the natural habitat of saline springs. However, since agricultural return flows are likely to be relatively constant, it is likely that populations of *S. ruficorne* are also relatively constant over time. In other words, this species is at worst a constant background issue, and outbreaks are not likely to be caused by this species (Palmer 2012, pers. comm.). Flow preferences of *S. chutteri* were consistent with the findings of Rivers-Moore et al. (2007).

Model development

Conceptual framework

An understanding of the dynamics of the problem, and how to address it, is best achieved using the conceptual flow-feeding model proposed by Palmer and Craig (2000). Using this model has the added advantage that should exact species not be available for laboratory experiments, equivalent species in terms of velocity/food availability groups can be used. Palmer and Craig (2000) proposed that seston availability was a major factor in the evolution of blackfly fan structure, and that particle concentration and water velocity were two of the most important determinants of blackfly larval distribution. Here, labral fan structure could be used to predict the habitat of blackfly species, with the model predicting four broad labral fan groups based on relative position along axes of water velocity and seston availability. Thus, *S. impukane* larval labral fan structures fall into the "weak complex" group (along with *S. merops* (W. Cape only) and *S. alcocki*, all members of the subgenus Pomeroyellum (de Moor 2003), preferring similar slow water velocities. Conversely, *S. chutteri* falls into the "strong porous" group for labral fan structure (diametrically opposite *S. impukane* – high velocities and high seston availability). There are no equivalent species, although the closest one (*S. bovis*) falls into the standard "middling" category, together with *S. damnosum*, with all three species preferring high velocities. *S. bovis* and *S. damnosum*, however, prefer lower seston levels (10-50 mg/l) to *S. chutteri* (> 50 mg/l) cf. *S. impukane*< 10 mg/l.

Four of the species group together in terms of the classification of Palmer and Craig (2000), preferring water with moderate (10-50 mg/L) seston levels, viz. *S. adersi*, *S. damnosum*,

S. mcmahoni and *S. medusaeforme*. Conversely, *S. chutteri* prefers seston-rich (>50 mg/L) water and high velocities, with *S. ruficorne* and *S. impukane* preferring the extreme opposite habitat (seston-poor, clear water: <10 mg/L, and slow flows). In this study, all species encountered generally had flow velocity preferences in agreement with the figures provided by Palmer and Craig (2000).

Based on the data from Palmer and Craig (2000), there is a clear relationship between larval morphology (number of posterior hooks) and flow velocity preferences (Figure 15). This reaffirms the relationship between species occurrence and flow conditions. A second axis for describing larval occurrence is seston size (turbidity) (Palmer and Craig 2000), such that the species from this study could be arranged in a matrix of flow velocity versus turbidity (Table 11). These data clearly illustrate that the dynamic relationship between discharge and turbidity will differentially favour different species of blackly at different times of the year under different flow and turbidity conditions.

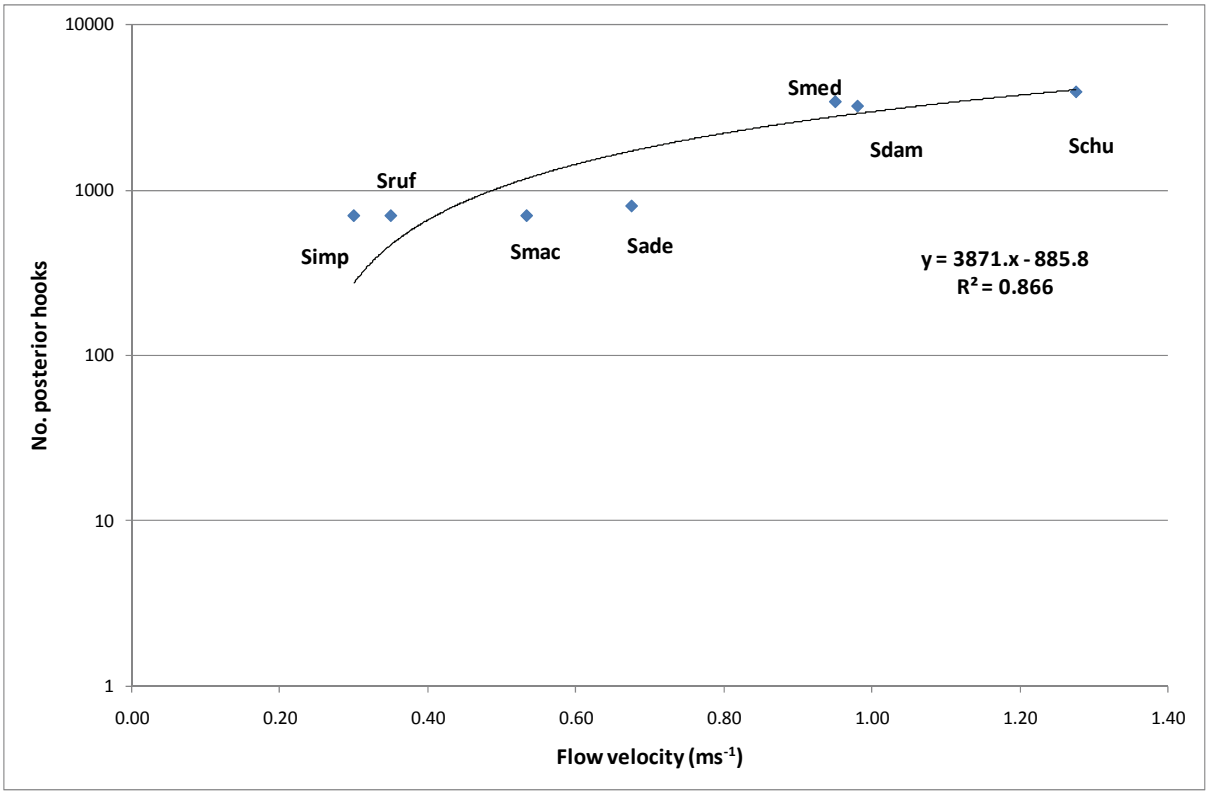


Figure 15 Number of larval posterior hooks versus flow velocity (based on data from Palmer and Craig 2000, with additions from Rivers-Moore et al. 2006 and from this study)

Table 11 Matrix of flow velocity versus turbidity showing relative preferences of different blackfly species sampled (after Palmer and Craig 2000)

	Flow velocity (ms^{-1})				
Turbidity (mg/L)	**<0.35**	**0.35-0.55**	**0.55-0.75**	**0.75-1.00**	**>1.00**
>50					Schu
10-50		Smac	Sade	Sdam, Smed	
<10	Simp				

A third habitat axis which remains to be understood is the relationship between water temperature regimes and thermal tolerances of the different blackfly species on the Orange River. From the very limited data collected in this survey, there are indications that water temperatures in the anastomosed channels exhibit higher diurnal variation than sites in the main channel. The most upstream site (main channel; UP6) had the least variable water temperatures, while the closest site to this in the anastomosed section (UP3) also had less variable temperatures. Sites within the anastomosed section exhibited relatively higher diurnal variation, as did the site receiving combined flows into the main channel (UP5). These short-term patterns were corroborated by the longer-term thermal time series from Marksdrift and Vioolsdrif, and confirmed that water temperatures are relatively stable from day to day, with the volume of water buffering temperatures. It is a reasonable hypothesis that species favouring lower flows may have either higher LT_{50} thresholds than *S. chutteri*, or is more tolerant of thermal variation, and therefore that during periods of low flows and lower turbidity, there is a stronger case for these species contributing towards outbreaks. The 24 h LT_{50} for *S. chutteri* was 26.1±1°C, in comparison to an ambient temperature of 30±1°C, which was lethal for both the larvae and the pupae of *S. nigritarse* (Begemann, 1980); and a critical thermal maximum of 30.3°C for unspecified species of Simuliidae (Dallas and Rivers-Moore, 2011).

Our thermal experiments suggest that egg development and hatching for *S. damnosum* was optimum at approximately 25°C, with a highly synchronous 100% hatch approximately nine days after oviposition. Percentage hatch was ≥ 99% at 10, 15 and 20°C, while hatching dropped to 74% at 30°C and failed completely at 33.5°C. This suggests a critical limit for development between 30 and 33.5°C. Begemann (1980) determined that eggs of both *S. nigritarse* and *S. adersi* took up to 13 days to hatch in water at a temperature of 25°C, while *S. chutteri* eggs took 4 to7 days to hatch at 25±1°C and 30±1°C, respectively, and 21 to 25

days at 10±1°C (Begemann 1986). In comparison, eggs of *S. damnosum* took approximately 9 days to hatch (estimated development of 5 days in the river, 1 day in transit and 4 days in the laboratory) at 25°C. In the case of *S. chutteri*, the duration of the aquatic stage varies between 12 and 45 days, depending on water temperatures (de Moor, 1982b, 1989). These hatching times appear to be correlated with flow velocity preferences (Figure 16).

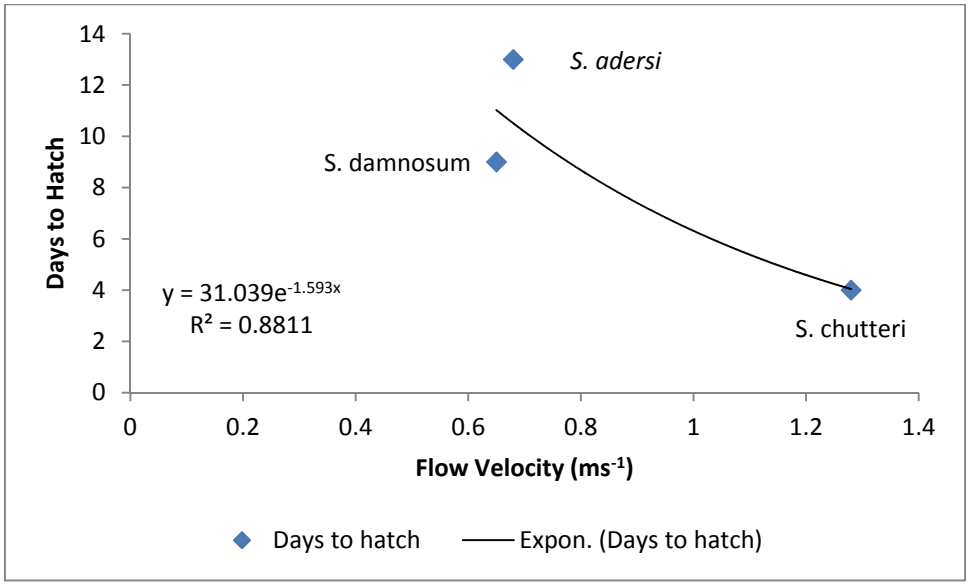


Figure 16 Relationship between flow velocity preference and days to hatching for three species of blackfly

Development of a Bayesian Network model

To integrate the two-dimensional model of seston and velocity preference for the species of blackfly in the Orange River with known variables controlling blackfly outbreaks (i.e. flow volumes and the functioning of the control programme), a simple Bayesian Network model was developed. Bayesian networks are particularly useful in predicting the likelihood of an event occurring based on conditional probabilities of causes. A Bayesian Network model essentially consists of cause-and-effect relationships, and is an ideal tool for knowledge representations for use in many situations involving reasoning and decision-making under uncertainty (Kjaerulff and Madsen 2008, p. 64). BNs provide a method of representing relationships between variables, even if the relationships involve uncertainty, unpredictability or imprecision (Batchelor and Cain 1999). They are also viewed as a strong tool for visualizing complexity and engaging stakeholders (Zorilla et al. 2010). Bayesian

analysis presents a way of dealing with subjective probabilities (i.e. prior probabilities) and how they may be updated based on new evidence. Conditional (posterior) probabilities are calculated from single or multiple prior probabilities and the accuracy of the prediction is a function of the reliability of the prior probabilities (Crilly 2007). Bayesian networks are relatively "forgiving" of poor input data, so that this approach is free from the arguments of "too little data" and BNs show good prediction accuracy even using small sample sizes (Uusitalo 2007).

For this model, causative variables of blackfly outbreaks were defined. Each variable was assigned two variable states. The probabilities of each state were defined based on a number of approaches, for example, for flow conditions the return intervals for flows below or exceeding 65 m^3s^{-1} were calculated from flow data for the Upington weir (D7H005). Quantitative data were unavailable for turbidity data, relative distances of single versus anastomosing channel sections, or periods of successful spraying, and so reasonable values were used instead (Table 12). Using these data, conditional probabilities of the blackfly species being *S. chutteri* or *S. impukane* (as a generic species representing low flow, low turbidity conditions typically occurring in the anastomosing sections of the middle Orange River) were generated based on combinations of parent node variable states (Tables 13-14).

Table 12 Variables, states and prior probabilities of the Bayesian Network model for blackfly outbreaks on the Orange River

Node	**States**	**Probabilities**
Turbidity	High, low	80 vs. 20%
Discharge	High, low	63 vs. 37%
Channel type	Single, anastomosing	90 vs. 10%
Simulium	chutteri, impukane	See CPT (Table 13)
Spraying	Successful, unsuccessful	80 vs. 20%
Outbreak Probability	High, low	See CPT (Table 14)

Table 13 Conditional probability table (CPT) for the likelihood of the problem species being either *S. chutteri* or *S. impukane*, based on combinations of the variables states for turbidity, discharge and channel type

Causative variables			Simulium	
Turbidity	**Discharge**	**Channel**	**impukane**	**chutteri**
Low	Low	Anas	1	0
Low	Low	Single	0.5	0.5
Low	High	Anas	0.6	0.4
Low	High	Single	0.3	0.7
High	Low	Anas	0.8	0.2
High	Low	Single	0.4	0.6
High	High	Anas	0.48	0.52
High	High	Single	0	1

Table 14 Conditional probability table (CPT) for the likelihood of a blackfly outbreak, based on combinations of the variables states for the type of blackfly species and the success of the spraying programme

		Outbreak Probability	
Simulium	**Spraying**	**Low**	**High**
impukane	Successful	100	0
impukane	Unsuccessful	90	10
chutteri	Successful	80	20
chutteri	Unsuccessful	0	100

When these data are combined into a Bayesian Network model, the results illustrate that under default probability conditions, there is a 28% probability of an outbreak at any time, assuming an 80% probability that the spraying programme is successful, and that *S. chutteri* is just over 3 times more likely to be the cause of the problem than *S. impukane* (Figure 17). With an observed effect of no outbreak (i.e. low outbreak probability = 100%: Figure 18), it is highly likely that the control programme is operating successfully (94.1%), while it is still more than twice as likely that residual species will be *S. chutteri* based on ambient flow, turbidity and channel conditions. As the model is currently configured, and where a confirmed outbreak is observed (i.e. outbreak probability = 100%; Figure 19), it is highly likely that the causative species is *S. chutteri* (98% probability), driven by high likelihoods of high flows, turbidities and where resident populations occur in the single channel areas. In this scenario, the probability of the spraying programme not being successful also contributes to the situation (56.3% probability of not being successful).

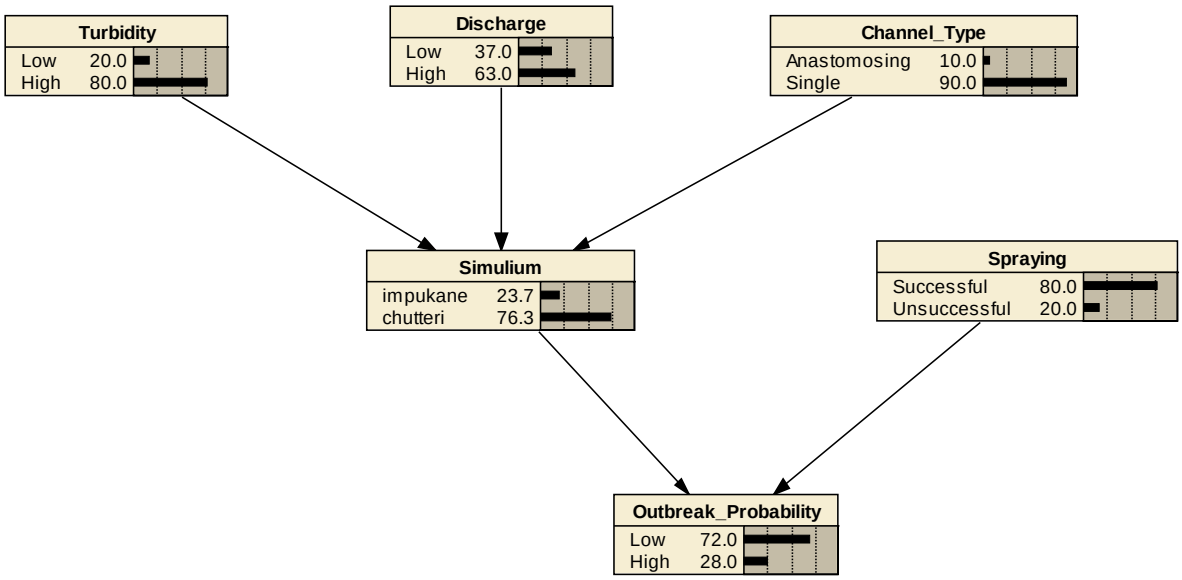


Figure 17 Bayesian Network diagram illustrating outbreak probability (observed effect) and the relative conditional likelihoods of the driver variables (*Simulium* species and success of the spraying programme) based on levels of turbidity, discharge and channel type.

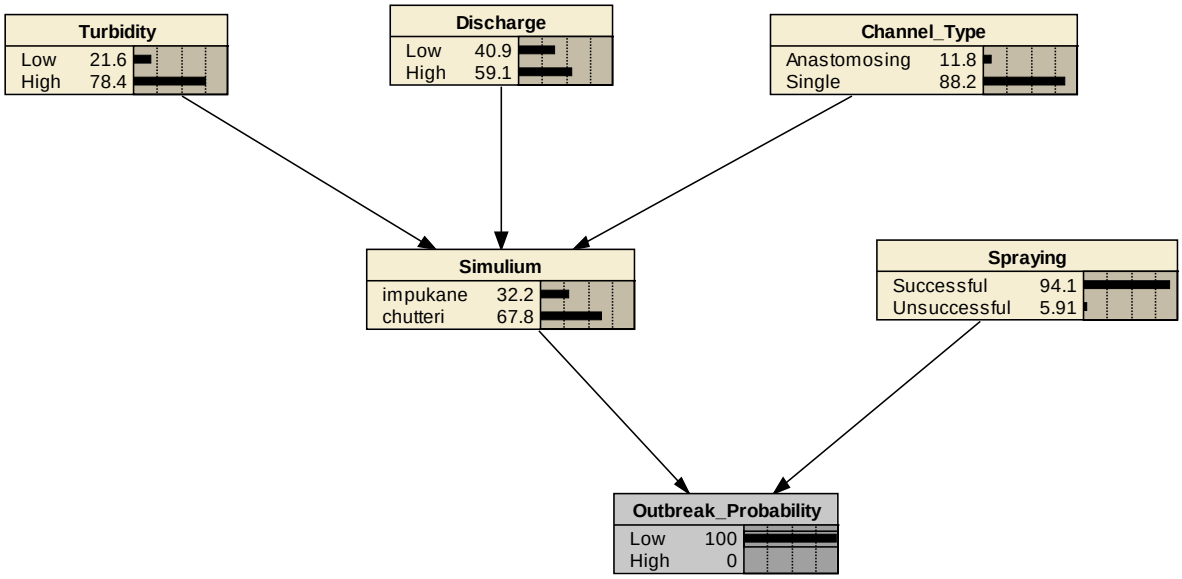


Figure 18 Bayesian Network diagram illustrating outbreak probability (where observed effect is a confirmed absence of outbreak) and the relative conditional likelihoods of the driver variables (*Simulium* species and success of the spraying programme) based on levels of turbidity, discharge and channel type.

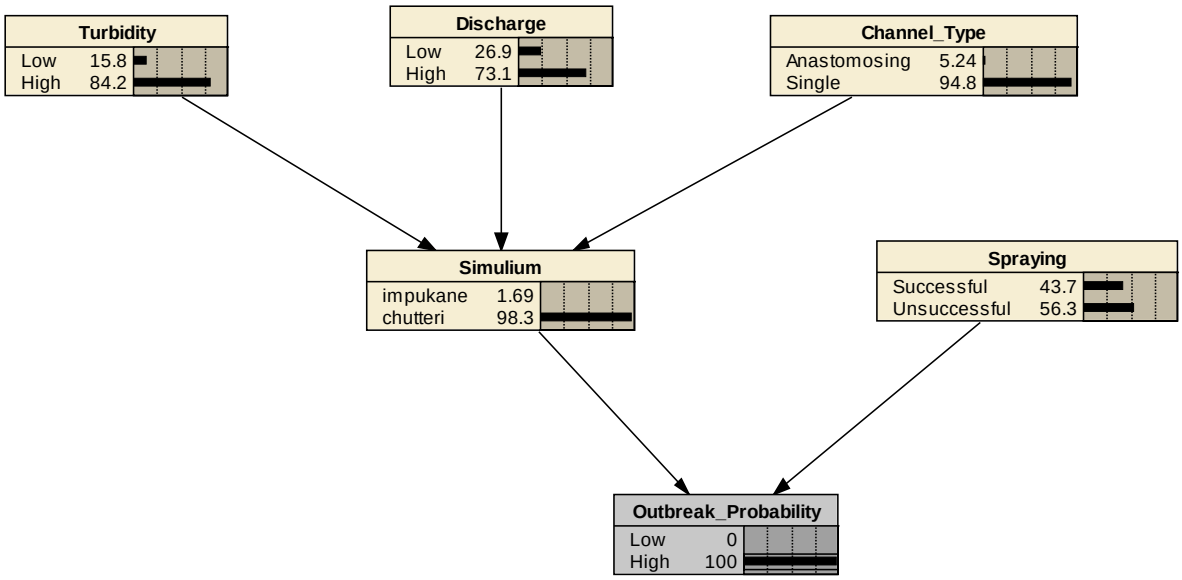


Figure 19 Bayesian Network diagram illustrating outbreak probability (where observed effect is a confirmed outbreak) and the relative conditional likelihoods of the driver variables (*Simulium* species and success of the spraying programme) based on levels of turbidity, discharge and channel type.

CONCLUSIONS

Based on a weight of evidence approach, it is unlikely that the outbreaks experienced during 2011 were the result of another species of blackfly additional to *S. chutteri*. Hydrologically, it is an anomaly that *S. impukane* is a problem in the Orange River. Given its larval habitat preferences, it was noted that there should always be some habitat for *S. impukane* along certain reaches of the Orange River. Optimal habitat conditions would be during low-flow periods of clear water; for example flows at Vioolsdrif (D8H009) periodically fall below 30 m^3s^{-1}.

At this stage, the Bayesian network model is a means of integrating the data examined in this study. Verification would still need to be undertaken on this model. However, already at this stage the model is useful because it highlights the need to collect turbidity data to refine the probabilities for that node, and the need to locate and characterise the anastomosing channels along the middle Orange River. Analyses of relationships between water quality and flow volumes, since outbreaks caused by species other than *S. chutteri* could be a problem related to recent unexplained changes in water quality (IBT)

Water temperature data indicate that there is certainly a basis for including temperatures as a third axis in the seston availability/velocity model, with indications that different species of blackfly exhibit different thermal preferences and sensitivities. The hypothesis that different thermal thresholds result in different outbreak behaviours of *S. chutteri* and *S. impukane* remains to be investigated. Such a study would be useful, given that Rivers-Moore et al. (2012) have already demonstrated the potential for different outbreak intensities based on relationships with water temperatures using a modelled the response of blackfly annual generation numbers to water temperatures. As a second hypothesis, given the different flow velocity preferences of *S. chutteri* versus *S. impukane*, it is proposed that differences in turbulence result in differences in feeding efficiencies of both species. This has implications both in terms of confirming preferred habitats and flow conditions of both species, and in how and where larvicide should be applied. Should future refinements of the Bayesian Network model be undertaken, there would be merit in adding temperature as a parent node, although further studies on the thermal tolerance of the different species would need to be undertaken.

Circumstantial evidence is that *S. impukane* was more a problem in braided sections (middle and lower) i.e. Upington and downstream, where there are more reeds. It is therefore most likely that the main cause of the blackfly problem remains *S. chutteri*, where sustained high flow volumes and turbidity levels favour this species over the other species of blackfly. However, during periods of lower flow and lower turbidity, other species of blackfly may be favoured and contribute towards periodic outbreaks. Additionally, anasotomosing reaches of the Orange River, such near Keimoes, which are difficult to apply larvicides to because of limited downstream carry, may act as reservoirs of various species of blackfly. This may contribute towards periodic outbreaks of pest blackfly, caused by multiple blackfly species including *S. damnosum* and *S. impukane*.

New control options are limited: Biological control of *S. chutteri* is not a viable option, particularly under high flows, as predator:prey ratios drop once flow velocities exceed 1 ms^{-1} (Rivers-Moore et al. 2007). Flow manipulation has potential, with certain caveats. Limited flow shutdowns have been shown to reduce blackfly larval densities without detrimental impacts on non-target macroinvertebrates (Rivers-Moore and de Moor 2008), and Rivers-Moore et al. (2008b) identified a flow threshold of 2 m^3s^{-1} in July for 38-40 days to achieve control in the Great Fish River. In the Orange River, flow reductions to< 35 m^3s^{-1} from Vanderkloof dam for 12 days in July, or Boegoeberg dam for 13 days with flows reduced to 2 m^3s^{-1}, have been recommended (Palmer et al. 2007). Winter flow manipulations are not a simple solution, however, and gains in blackfly control unfortunately compete with other economic priorities, for example, one problem is that there is a clash with Eskom's winter flow generation period. What this study has highlighted is that the best avenues for improved blackfly control should focus on the following two areas:

1. Management issues around the control programme itself, as previously highlighted by Palmer et al. (2007)
2. Hydraulic studies linked to investigations of carry in the anastomosing sections, which are likely to be the least well controlled areas on the middle Orange River. However, while it is unfortunate that these sections are associated with urban areas, it is also important to bear in mind that residual populations of different species of blackfly should be maintained in the range river as parts of its natural ecological functioning, and that the anastomosing sections could be serving a role as refugia.

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Appendix I: List of Workshop attendees

Ten people attended the expert workshop, who were invited based on their involvement in the supply and application of larvicide, as well as blackfly larval density monitoring (Table 1).

Table 1 List of attendees at expert workshop to assess gaps in blackfly control programme

Name	Organisation	Email	Tel	Role/ Experience
Gert Greyvenstein (GG)	NDA[1]	gertg@nda.agric.za	053-6313122	De Aar office. Involved in blackfly control programme since 1995.
Ian Garden (IG)	Philagro/ValentBioScience	ian.garden@philagro.co.za	0828056667	Technical marketing manager with Philagro. Involvement in blackfly control programme (monitoring and spraying; supply of product on tender) dates back to 1995 (originally with Abbot Labs);
John Mansfield (JM)	Philagro	john.mansfield@philagro.co.za	012-3488808	Liaison; limited direct involvement
John Tladi (JM)	NDA	johntl@nda.agric.za	012-3197568	NDA, Pretoria
lKalafeng Ben Kgakatsi (BK)	DAFF[2]	lkalafengk@daff.gov.za	012-3197955/6	Risk management/climate change
MatigaMotsepe (MM)	DAFF	matigam@daff.gov.za	012-3196768	Risk management/climate change
Nick Rivers-Moore (NRM)	Independent	blackfly1@vodamail.co.za	033-3433807	Blackfly research on Orange and Great Fish Rivers (2004-2007)
NthabisengKgotla (NK)	NDA	nthabisengm@nda.agric.za		Upington office; Involvement in BCP since March 2012 (weekly monitoring)
Rob Palmer (RP)	Nepid	rob@nepid.co.za	013-7511533	Developed original WRC-funded blackfly larval control programme over a six-year period (1992-1997) based in Upington
VuyokazimPuluana (VM)	NDA	vuyok@nda.agric.za	053-6313621	De Aar office; Involvement in BCP for past 3 years (managing of control and monitoring)

[1] National Department of Agriculture; [2]Department of Agriculture, Forestry and Fisheries

Appendix II Additional Flow Analyses for two weirs on the middle Orange River

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Context

Hydrological alteration within a river network can be defined as a change in the natural flow of a river as a result of a perturbation such as a dam or inter basin transfer scheme (Richter et al. 1996), which can have significant effects on the native riverine biota (Richter et al. 1998). The main causes of hydrological alteration in the Orange River Catchment are believed to result mainly from the construction of the Gariep and Vanderkloof Dams as well as the inter-basin transfer scheme from the Orange River to the Great Fish River (Rivers-Moore et al. 2007). Gariep Dam was constructed in 1971 and Vanderkloof Dam was constructed in 1977 respectively (DWAF 2009). The constructed dams result in a discontinuity of the river system and have significant implications for the seasonal variation of flow and the duration of high and low flow periods. The aim of these analyses was to investigate the effects of hydrological alteration on the stream flow characteristics of the Middle and Lower Orange River Basin.

The ecological basis for this routine is that different characteristics of the natural streamflow regime regulate the ecological functioning and that these streamflow characteristics are described by statistical measures as “ecologically relevant hydrological indices” (Richter et al. 1996). Using the IHA together with the “Range of Variability” approach, the IHA method can identify periods of incompatibility between ecological and societal freshwater needs and be used to set water management targets and can consequently describe baseline flows for rivers where the primary management objective is the protection of the natural ecosystem functioning (Richter et al. 1997). The IHA method characterizes within-year variation in streamflow on the basis of a series of hydrologic attributes organized into five groups which describe flows in terms of frequency, duration, timing and magnitude of flow events. Alterations to the streamflow regime indicated by changes in these statistics provides insights into temporal changes in flows patterns, which are of use in developing restoration criteria (Richter et al. 1998).

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Flow analysis of D7H008 (Boegoeberg)

Table A1 Statistics for the five Indicators of Hydrologic Alteration flow group types defined by Richter et al. (1996) for D7H008. Pre- and post-impact periods were defined as 1933-1975 (42 years) and 1976-2012 (37 years) respectively

	MEANS		COEFF. of VAR.		DEVIATION FACTOR		DEV. of C.V.	
	Pre	Post	Pre	Post	Magnitude	%	Magnitude	%
Parameter Group #1								
October	160.6	151.3	2.128	1.033	-9.3	-5.792	-1.095	-51.47
November	325.9	204.6	1.307	0.9397	-121.2	-37.21	-0.367	-28.09
December	376	243.6	1.148	1.117	-132.4	-35.22	-0.03084	-2.688
January	361.3	314.8	1.294	1.809	-46.51	-12.87	0.515	39.81
February	612.4	493.9	1.072	1.481	-118.5	-19.35	0.4089	38.15
March	518.7	491.3	0.9582	1.756	-27.45	-5.291	0.7975	83.23
April	361.9	330.9	0.8715	1.33	-30.91	-8.543	0.459	52.67
May	228.3	211.8	1.174	1.412	-16.49	-7.221	0.2384	20.31
June	134	180.6	1.049	1.705	46.62	34.79	0.6562	62.54
July	77.84	113.5	1.062	0.9646	35.66	45.81	-0.09771	-9.198
August	59.86	101.9	1.242	1.002	42.01	70.19	-0.2406	-19.37
September	106.5	125.1	1.707	1.131	18.56	17.43	-0.5757	-33.73
Mean |%| change						25		36.8
Parameter Group #2								
1-day minimum	11.05	30.65	0.9349	0.6874	19.6	177.3	-0.2474	-26.47
3-day minimum	12.59	32.76	0.9199	0.6692	20.16	160.2	-0.2507	-27.25
7-day minimum	14.69	37.05	1.008	0.6526	22.36	152.2	-0.3558	-35.28
30-day minimum	21.67	50.7	1.182	0.5909	29.03	134	-0.5916	-50.03
90-day minimum	50.58	80.31	0.9817	0.7777	29.73	58.77	-0.204	-20.78
1-day maximum	2658	1394	0.6332	1.293	-1264	-47.55	0.6598	104.2
3-day maximum	2345	1337	0.6771	1.29	-1007	-42.97	0.6128	90.5
7-day maximum	1903	1217	0.664	1.285	-686.2	-36.05	0.6209	93.52
30-day maximum	1153	845.3	0.6523	1.365	-307.2	-26.66	0.7123	109.2
90-day maximum	670.7	537	0.6748	1.245	-133.7	-19.93	0.5699	84.46
Number of zero days	0.2326	0	6.557	0	-0.2326	-100	-6.557	-100
Base flow index	0.05891	0.2406	0.9008	0.5913	0.1817	308.4	-0.3095	-34.36
Mean |%| change						105.3		64.7
Parameter Group #3								
Date of minimum	240.7	221.3	0.2404	0.1747	19.37	10.59	-0.06578	-27.36
Date of maximum	43.51	37.28	0.2173	0.2015	6.234	3.406	-0.01577	-7.257
Mean |%| change						7		17.3
Parameter Group #4								
Low pulse count	6.209	4.5	0.755	0.9643	-1.709	-27.53	0.2092	27.71
Low pulse duration	17.13	6.107	0.9237	0.8783	-11.02	-64.35	-0.04538	-4.912
High pulse count	4.186	1.222	0.6475	1.465	-2.964	-70.8	0.8176	126.3
High pulse duration	9.428	19.7	1.168	1.233	10.28	109	0.06564	5.621
Low Pulse Threshold	31.48							
High Pulse Threshold	772.8							
Mean |%| change						67.9		41.1
Parameter Group #5								
Rise rate	86.34	33.33	0.5478	0.8676	-53.01	-61.4	0.3198	58.38
Fall rate	-47.6	-26.27	-0.4577	-0.8276	21.33	-44.81	-0.3699	80.82
Number of reversals	109.1	125.3	0.2757	0.2455	16.16	14.81	-0.0302	-10.96
Mean |%| change						40.3		50

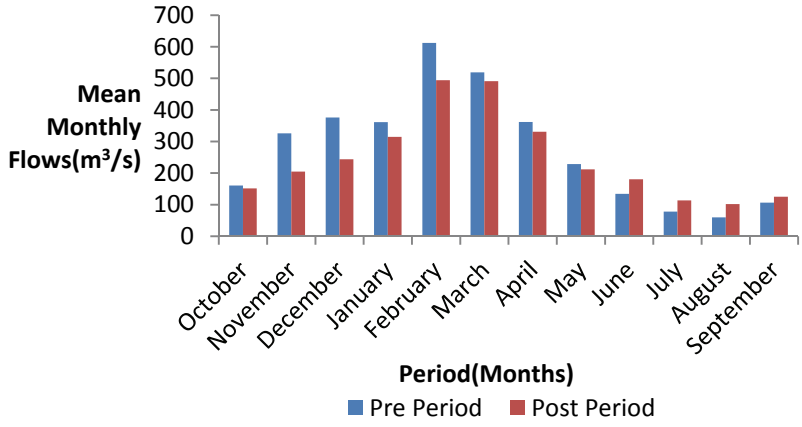

Figure A1 Bar graph showing the mean monthly flows for the pre- and post-impoundment periods for weir D7H008. Mean annual flows for both periods were 274.8 m^3s^{-1} and 245.7 m^3s^{-1} respectively.

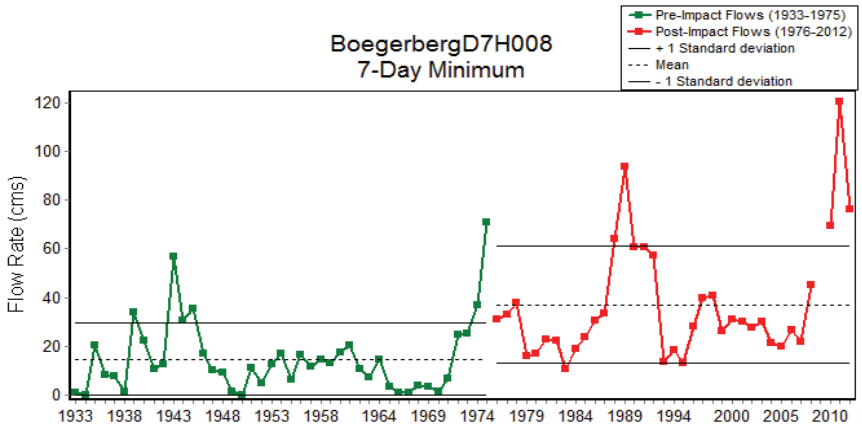

Figure A2 Graph showing the 7–Day Minimum flows for D7H008 for the pre- and post-impoundment period.

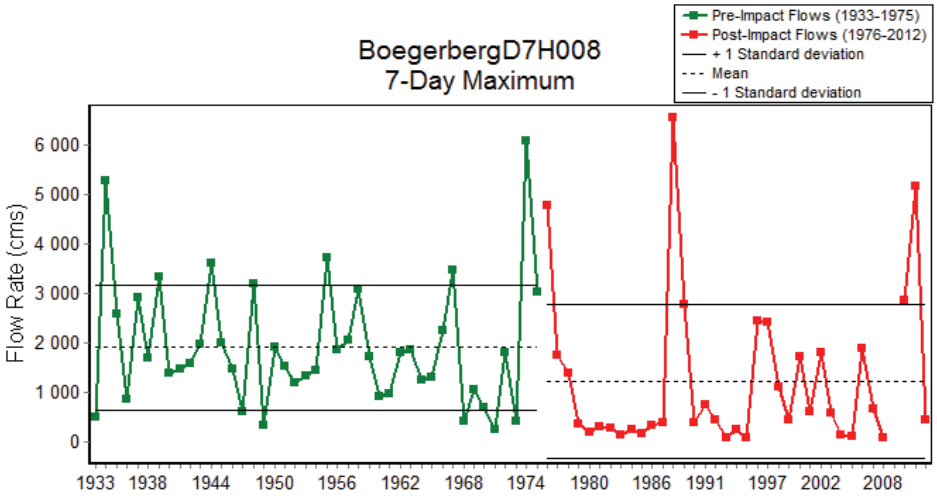

Figure A3 Graph showing the 7–Day Maximum flows for D7H008 for the pre- and post-impoundment period.

Flow analysis of D8H003 (Vioolsdrif)

Table A2 Statistics for the five Indicators of Hydrologic Alteration flow group types defined by Richter et al. (1996) for D7H008. Pre- and post-impact periods were defined as 1936-1975 (40 years) and 1976-2012 (37 years) respectively

	MEANS		COEFF. Of VAR.		DEVIATION FACTOR		DEV. Of C.V.	
	Pre	Post	Pre	Post	Magnitude	%	Magnitude	%
Parameter Group #1								
October	212.5	106.9	3.275	1.128	-105.6	-49.7	-2.148	-65.57
November	332.9	146.2	1.543	1.086	-186.7	-56.07	-0.4571	-29.62
December	396.8	206.1	1.388	1.328	-190.7	-48.07	-0.0604	-4.351
January	356	263.7	1.05	1.771	-92.27	-25.92	0.7203	68.58
February	733.3	379.9	1.127	1.583	-353.4	-48.19	0.4563	40.49
March	610.9	441.9	0.9218	2.03	-169	-27.67	1.109	120.3
April	509.7	281.2	0.8955	1.179	-228.5	-44.83	0.2831	31.61
May	331.3	200	1.229	1.257	-131.3	-39.64	0.02816	2.291
June	168.6	159	1.09	1.479	-9.619	-5.705	0.389	35.68
July	87.58	114.2	1.286	0.9393	26.65	30.43	-0.3467	-26.96
August	69.42	88.88	1.511	0.9475	19.45	28.02	-0.5637	-37.3
September	97.74	104.2	2.274	1.396	6.42	6.568	-0.8783	-38.62
Mean |%| change						34.2		41.8
Parameter Group #2								
1-day minimum	4.285	13.06	2.79	1.559	8.771	204.7	-1.231	-44.13
3-day minimum	4.792	13.99	2.884	1.491	9.198	192	-1.393	-48.31
7-day minimum	5.748	16.48	3.031	1.422	10.73	186.7	-1.609	-53.08
30-day minimum	11.75	30.53	2.727	1.143	18.78	159.7	-1.584	-58.07
90-day minimum	46.02	61.99	1.541	1.055	15.97	34.69	-0.4867	-31.58
1-day maximum	2786	1226	0.6061	1.306	-1560	-55.99	0.6997	115.4
3-day maximum	2543	1186	0.6296	1.295	-1357	-53.36	0.6651	105.6
7-day maximum	2172	1087	0.6943	1.287	-1085	-49.94	0.5932	85.43
30-day maximum	1374	783.7	0.7167	1.402	-590.1	-42.95	0.6849	95.55
90-day maximum	807.2	467.2	0.706	1.182	-339.9	-42.12	0.4762	67.46
Number of zero days	22.65	0.2703	1.798	6.083	-22.38	-98.81	4.285	238.4
Base flow index	0.02183	0.1004	3.281	1.008	0.07857	359.9	-2.273	-69.28
Mean |%| change						123.4		84.4
Parameter Group #3								
Date of minimum	252.9	305.1	0.2355	0.2407	52.13	28.49	0.005158	2.19
Date of maximum	51.05	55.24	0.215	0.2076	4.193	2.291	-0.007361	-3.424
Mean |%| change						15.4		2.8
Parameter Group #4								
Low pulse count	3.275	5.135	0.6739	1.033	1.86	56.8	0.3587	53.23
Low pulse duration	35.22	7.875	1.071	0.8737	-27.34	-77.64	-0.1975	-18.43
High pulse count	2.725	0.9459	0.8014	1.813	-1.779	-65.29	1.012	126.2
High pulse duration	11	12.62	0.7201	0.4313	1.62	14.74	-0.2888	-40.11
Low Pulse Threshold	14.18							
High Pulse Threshold	938.9							
Mean |%| change						53.6		59.5
Parameter Group #5								
Rise rate	83.52	28.51	0.5753	0.8195	-55.01	-65.86	0.2443	42.46
Fall rate	-36.07	-17.53	-0.6482	-0.8403	18.55	-51.41	-0.192	29.62
Number of reversals	66.25	97.51	0.3807	0.1493	31.26	47.19	-0.2314	-60.78
Mean |%| change						54.8		44.3

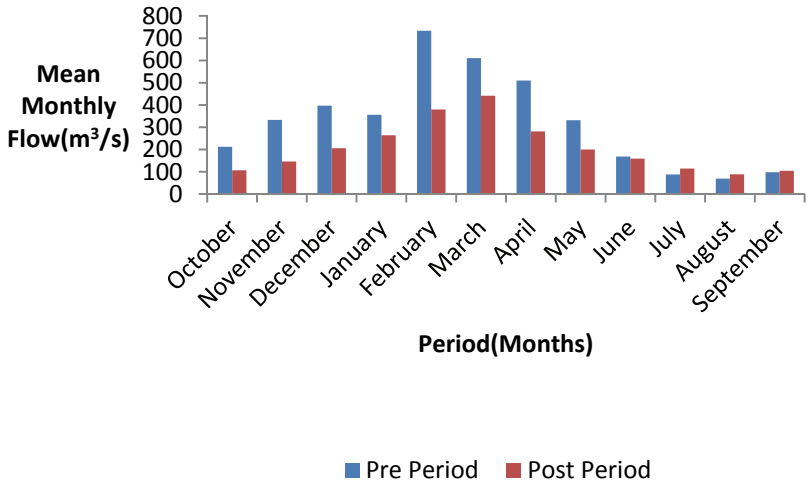

Figure A4 Graph of mean monthly flow for weir D8H003. Mean annual flows for both periods were 322.8 m^3s^{-1} and 206.8 m^3s^{-1} respectively.

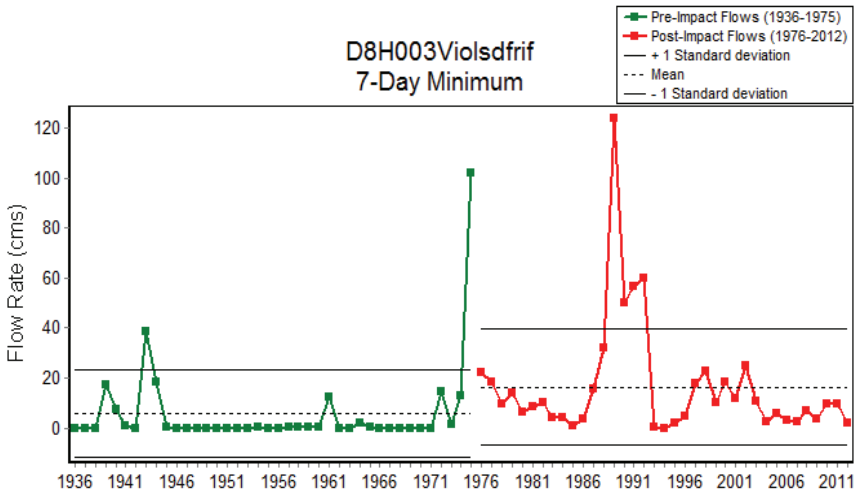

Figure A5 Graph showing the 7–Day Minimum flows for D8H003 for the pre- and post-impoundment period.

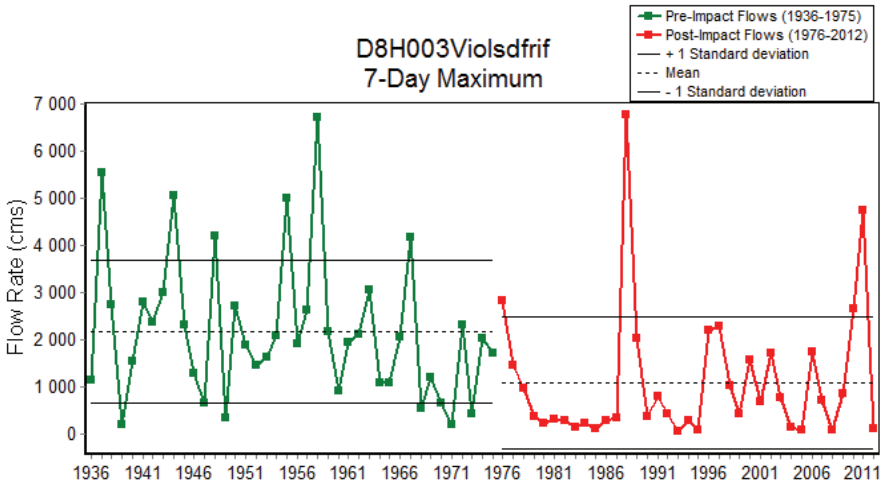

Figure A6 Graph showing the 7–Day Maximum flows for D8H003 for the pre- and post-impoundment period.