Canadian Journal of Zoology
Revue canadienne de zoologie

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Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2017-0285.R1
Manuscript Type:	Article
Date Submitted by the Author:	05-Dec-2017
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Keyword:	benthic algae, black fly, black fly control, Simulium damnosum, Simulium chutteri, water temperature, system switch

The influence of turbidity and water temperature on black fly species in the middle and lower Orange River, South Africa

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Abstract

Water clarity in the Orange River of South Africa alternates between highly turbid and clearer conditions. Multiple ecological effects result from this that affect aquatic community structure and species composition, including switching of dominant black fly species and growth of benthic algae. Fourteen sites in the middle and lower Orange River were sampled for black fly species and abundances, presence of benthic algae, water quality and turbidity seasonally between November 2015 and December 2016. Logistic regression models, time series analyses and multivariate ordinations showed that clearer water favours benthic algae and the minor pest black fly species *Simulium adersi* Pomeroy, 1922 and *S. nigritarse* Coquillett, 1902, while high turbidity favours the major pest black fly species *S. chutteri* Lewis, 1965 and *S. damnosum* Theobald, 1903. Moreover, these switches appear to be less frequent under post-impoundment conditions. We conclude that black fly species composition is dynamic and reflects changes in flow and turbidity levels. Flow regulation has resulted in a trade-off between improved water supply and ecological costs resulting from reduced ecosystem variability.

Keywords: benthic algae; black fly; black fly control; *Simulium damnosum*; *Simulium chutteri*; system switch; water temperature

Introduction

Alternative stable states have been recognised as a theory explaining abrupt changes in ecological systems for almost fifty years (Holling 1973), where ecosystem states change abruptly in response to exceedance of ecological thresholds. Such switches are distinct from gradual linear changes typical of natural systems, versus abrupt catastrophic switches

triggered by key variables in systems where resilience has been compromised (Scheffer et al. 2001). Examples of systems existing under alternative stable state regimes are known from various ecosystems including lakes and lotic systems (Scheffer et al. 2001). Typically, switching between states is unpredictable and triggered by stochastic events; however, their dynamic can have implications for periodic pest outbreaks and the degree of success of control programmes (Schooler et al. 2011). One such example of a river system alternating between clear and turbid conditions is the Orange River of South Africa.

The Orange River has a unique place in South Africa's bio-geographical, hydrological and political histories, as the oldest, longest and biggest (by volume) river system in the country (Skelton 1986; van Vuuren 2012). The river is a rich water resource in the driest part of South Africa, where the stone-age implements and place names reflect human use going back for millennia. Progressive impoundment of the middle and lower river reaches between the 1930s and 1970s has provided access to this resource, but has also increased the incidence of outbreaks of pest black flies (Rivers-Moore et al. 2014). The major controlling impoundment for flows on the Orange River is the van der Kloof Dam, which became operational in 1977. With flow time series data going back to the 1940s, hydro-ecological studies can consequently be divided, where relevant, into pre- and post-impoundment periods with 1977 as the dividing event.

Riffles, rapids and cascades provide the hydraulic habitat for species of naturally occurring black fly with preferences for high flow velocities and high turbidity. A scientifically-based control programme using helicopter applications of two types of larvicides was initiated in 1991, and continues to date. However, periodic outbreaks of black fly occur, with the most recent outbreak in 2011 (Rivers-Moore et al. 2014), and before that in 2000-2001 (Palmer et al. 2007). The control programme extends over some 850 km of the middle and lower Orange River, where 250 rapids provide optimal breeding habitat for pest black fly species (Palmer et al. 2007). The success of the control programme depends largely on correct timing of larvicide applications. Black fly outbreaks along the middle and lower Orange River have the potential to cause losses to livestock production estimated conservatively at US\$13.3 million per annum (Rivers-Moore et al. 2014), and this excludes losses in the tourism and irrigated agricultural sectors, primarily through high annoyance levels (Mullins

2007). The major pest species are *Simulium chutteri* Lewis, 1965 and *S. damnosum* Theobald, 1903, but *S. nigritarse* Coquillett, 1902 and *S. adersi* Pomeroy, 1922 are also culprits (de Moor 1994, and citing others). Adult females of *S. chutteri* and *S. damnosum* feed primarily on mammals (livestock), whereas *S. nigritarse* and *S. adersi* feed primarily on birds.

All four of the main black fly species display varying degrees of generalised, altricial life history patterns with several gonotrophic cycles per annum and similar potential fecundities, making these species able to thrive with regulated flows (Brittain 1991; de Moor 1989; Palmer 1997). Their bionomics are all characterised by strategies of asynchronous development, flexible voltinism, and comparative insensitivity to temperature change, rapid colonisation ability, and high mobility (de Moor 1994). Consequently, larvae are present throughout the year, with habitat suitability attaining its highest value when abiotic influences (hydraulic habitat, water temperature and turbidity) are optimum, and biotic influences (predation and benthic algae) at a minimum (de Moor 1994). A combination of differences in life history strategies and labral fan structure determine which species prevail under particular environmental conditions. Eggs of all species mentioned above are laid as patches on stones or trailing vegetation, except *S. chutteri*, which scatter eggs in pools upstream of riffles. This behaviour is regarded as more primitive than laying the eggs on substrates, but it gives *S. chutteri* a competitive advantage in rivers with regulated flows, as this trait reduces the risks of eggs drying.

Hydraulic preference data support the assertion that the main cause of the black fly problem remains *S. chutteri*, where sustained high flow volumes and turbidity levels favour this species over the other species of black fly (Rivers-Moore et al. 2007). However, during periods of lower flow and lower turbidity, other species of black fly tend to be more abundant. Changes in turbidity cause switches in black fly species composition and concomitant changes in abundance of benthic algae, and this was also noted in the Vaal River by de Moor (1994). Benthic algae typically occur in low turbidity conditions, and their presence decreases the suitability of rocky substrate for black fly larval attachment. Switching in dominance of black fly species in response to changes in flow volumes and turbidity has also been reported in other large river systems (Fredeen 1977). A dynamic

spatio-temporal hydraulic habitat template where water levels affect turbidity levels is likely to underpin such switches. Here, the river system will be composed of a mosaic of habitat patches favouring different biotic elements at any one moment (Townsend 1989). Water temperatures also influence abundance of benthic algae (de Moor 1994), and rate of black fly larval development (de Moor 1982, 1994).

Palmer and Craig (2000) proposed that seston concentration and water velocity were major factors in the evolution of black fly labral fan structure, and two of the most important determinants of black fly larval distribution. Accordingly, labral fan structure could be used to predict the distribution of black fly species, with the model predicting five broad labral fan groups based on relative position along axes of water velocity and seston availability. In the Orange River, the major pest black fly (*Simulium chatteri*) falls into the “strong porous” fan type, with preferences for relatively higher seston concentrations and higher flow velocities. By contrast, the pest black fly *S. damnosum* and species that feed on birds (*S. nigritarse*, *S. adersi* and *S. mcmahoni* de Meillon, 1940) fall into the “standard” fan type group, with preferences for moderate seston levels of 10-50 mg/L over a range of velocities. The black fly species *S. impukane* de Meillon, 1936 and *S. ruficorne* Macquart, 1838 fall into the “weak complex” fan type, with a preference for clear water. Considerable research has been undertaken on factors influencing black fly populations (for example, Carlsson 1967; Fredeen 1977; de Moor 1994; Rivers-Moore et al. 2007), but what remains unknown are the ecological responses to system switches between turbid and clear water conditions. The aims of this study were firstly, to test the hypothesis that hydraulic conditions under which the middle and lower Orange River switches between clear and turbid conditions has no influence on black fly species composition; and secondly, to evaluate the potential of biological control through predation of pest black fly under turbid conditions using potential predator: prey ratios (hydropsychid predators versus *S. chatteri* larvae).

Methods

Study sites and data collection

Fourteen sites distributed along some 600km downstream of van der Kloof Dam, across the black fly problem area on the middle and lower Orange River, were selected for this study (Table 1). These sites extended over an elevation range of some 600m. Sampling was

undertaken in late spring (November 2015), late summer (March 2016), winter (July 2016), and early summer (December 2016). Turbidity data (cm) were collected using a clarity tube (Kilroy and Biggs 2002). Three spot readings of pH and conductivity ($\mu\text{S}/\text{cm}$) were recorded and averaged for each site during each survey, using a Hanna pH/conductivity meter. Water temperature data were collected at 13 sites, using Hobo TidBit v2 water temperature loggers, at hourly intervals between 4 November 2015 and 2 November 2016.

At each site black fly larvae and pupae were sampled across a range of hydraulic habitats. Samples were either collected from reeds, which were cut into short lengths and preserved, or collected from rocks in-current, using a 250 μm -mesh net held downstream while rocks were kicked and brushed. Larval densities were rated according to the 10-point scale of Palmer (1994). Black fly pupae and larvae were collected and preserved in 70% ethanol. The presence or absence of benthic algae was recorded from site observations at each field survey, and sites with high numbers of the density-independent black fly predator *Cheumatopsyche* spp. (Trichoptera: Hydropsychidae). Hydropsychid abundances were estimated based on habitat area measured on-site, and density calculated from photographs.

Analyses

Site groupings based on turbidity, pH and conductivity were assessed using a Principal Components Analysis (correlation matrix) (McCune and Mefford 2011), and incorporating categorical site scores for benthic algal presence or absence. For the water temperature time series, data were summarized into daily minima, maxima and means. Thermal seasons were divided based on observed mean daily water temperatures using regime shift detection software (Rodionov 2006; $p < 0.01$; cut-off length = 30; Huber's weight parameter = 1).

Numbers of individuals per species for each site and sampling period were recorded, with black flies in each sample identified to species using the taxonomic keys in de Moor (2003). Species were grouped according to the seston concentration-velocity preference model of Palmer and Craig (2000; Figure 1). Raw abundance data per species and life history stage were used in analyses, as well as presence/ absence and percentage data.

Black fly species turnover between seasons and sites was compared using a Bray-Curtis analysis (Sørensen (Bray-Curtis) distance measure; McCune and Mefford 2011), with site groupings defined using a cluster analysis (Sørensen (Bray-Curtis) distance measure, Group Average linkage method; McCune and Mefford 2011). Orders of magnitude between abundance of black fly and hydropsychid predators, abundances of each were plotted according to Palmer's (1994) 10-point method of scoring black fly larval density. Here, it was assumed that hydropsychid numbers would reach an asymptote due to available habitat for webs. The ratio of black fly (prey) to hydropsychid (predator) abundance was calculated and plotted.

To characterise likely response of species to changes in turbidity, the probability of occurrence of all sampled black fly species was modelled using simple logistic regression in the statistical software R (R Development Core Team 2009). Water clarity was used as a predictor variable, and presence/ absence of each species as the response variable. Similarly, the probability of occurrence of benthic algae was modelled using multiple logistic regressions, with water clarity and black fly abundance (density scores and total abundance of *S. chutteri* and *S. damnosum* larvae) as predictor variables and presence/ absence of benthic algae as the response variable. Data from all sites and all surveys were combined for these models.

To allow comparison with the fan-complex model, turbidity values were converted to seston concentrations (mg/L), according to Equation 1 (Palmer 1997; Rivers-Moore et al. 2007). Next, we needed long-term time series of seston concentration, and Palmer (1997) identified an exponential relationship between flow rates and seston concentration (Equation 2). Observed mean daily flow data time series were obtained from the South African Department of Water Affairs and Sanitation Hydrological Information System (www.dwaf.gov.za/Hydrology). Time series of mean daily seston concentrations were generated from the mean daily flow data at Upington (weir D7H005; 1942-current). Return intervals were calculated for a threshold of 30 mg/L (31 cm clarity), which was chosen as a median value for the "standard" fan type. Using this threshold, we calculated the number of potential switching events for pre- and post-impoundment time periods. We defined a switching event as being when seston concentration was < 30 mg/L for > 12 days, on the

basis that this is the minimum period required for *S. chutteri* to complete its aquatic life stage (de Moor 1994). Thus, with each successive day where seston concentration is < 30 mg/L, the probability of hydraulic habitat switching from turbid to clear increased additively by a factor of 1/12. We enumerated the events per month for pre- and post-impoundment periods (1942-1977 and 1978-2016). The duration of each event was calculated from the number of successive days where the event probability was one.

$$TSS = \exp\left(\frac{\log\frac{SD}{256}}{-0.616}\right) \quad [1]$$

$$Seston = 1.92 * Flow^{0.755} \quad [2]$$

where *TSS* is Total Suspended Solids (mg/L) and *SD* is clarity (cm)

Results

Turbidity, conductivity and pH data generally exhibited seasonal differences, whereas spatial changes in the variables measured were negligible. Values of pH generally reflected neutral to slightly alkaline conditions at all sites (7.0-8.5). Conductivity values ranged from 400-600 $\mu\text{S}/\text{cm}$, with the exception of Site 8b, where values were between 600 and 1500 $\mu\text{S}/\text{cm}$. Conductivities were highest during the July 2016 survey, corresponding with relatively lower flow volumes. There was a clear distinction between sites where benthic algae dominated (Figure 2; Table 2), versus sites where algae were absent. Separation of sites in terms of water quality was explained by a pH gradient (Axis 2), and sites with benthic algae present being associated with more alkaline pH values. Hourly water temperatures exhibited considerable homogeneity between sites, with a marked reduction in daily water temperature ranges and a cooling trend from late March until August. For example, average annual water temperatures were 20.5 ± 5.2 °C at Site 9, with absolute minima and maxima being 10.7 and 29.8 °C respectively. The annual mean temperature for Sites 1 and 2 were cooler by 1 °C. The annual thermograph showed ten significant changes in mean daily water temperatures (Figure 3).

Seven species of black fly were sampled over the course of the study, with the two most dominant species being *S. chutteri* and *S. damnosum* (Table 3). Relative proportions of contribution to overall sample numbers showed switching of dominant species between sites and seasons, but with specific site clusters based on species abundances, and showed

clear switching of dominant species between sites and seasons (Figure 4). *Simulium chatteri* and *S. damnosum* showed a negative association (Figure 5), with the former dominating in November 2015 and March 2016, and the latter being dominant in July and December 2016. Predatory hydropsychid trichoptera occurred in high abundance at Sites 7 and 8 when water clarity exceeded 40 cm. Densities of hydropsychids were approximately 20 individuals per 10 cm², so that for the 9x1 m ridge at Site 7, there was maximum potential habitat of $\leq 180\,000$ hydropsychid (total area of 90 000 cm² = 9 000 habitat units of 10 cm²). Hypothetical plots of potential abundances of hydropsychid and pest black fly using Palmer's (1994) abundance rating system, as well as prey: predator ratios indicate that above a density class of 6, predation effects of hydropsychid on pest black fly become increasingly irrelevant (Figure 6).

Water clarity was a strong predictor of probability of occurrence for five of the seven black fly species sampled, as well as for benthic algae (Figure 7; Table 4). Models indicated that at high turbidity *S. chatteri*, and less so *S. damnosum*, were more likely to occur. Conversely, as water clarity increased, the major pest species are less likely to occur, while the probability of occurrence of *S. adersi*/ *S. nigritarse* increased. System switching between black fly species groups and dominance of benthic algae occurred in the region of 60 cm water clarity (≈ 11 mg/L seston concentration). The probability of benthic algae was co-dependent on both clarity and black fly presence.

The number of potential switching events was more than four times higher for the pre-impoundment period than for the post-impoundment period (39 versus nine events) (Figure 8). The probability of occurrence was similarly three times higher for pre-impoundment conditions than for post-impoundment conditions ($p = 0.19$ versus $p = 0.06$), or an average of 1.11 events per annum versus 0.24 events per annum. Event duration was less for post-impoundment than for pre-impoundment flows (48 ± 39 versus 36 ± 38 days), but because of high standard deviations for both periods of analysis, duration differences were non-significant (Student's *t* test; $p > 0.05$). The majority of events occurred in spring (September to November; Figure 3).

Discussion

Conductivity and pH were relatively consistent across the 600 km study axis, with the exception of peripheral habitat that caters for different black fly species with specific water quality preferences. High ($> 1000 \mu\text{S}/\text{cm}$) conductivities in the irrigation return flow channels provided favourable habitat for the halophilic *S. ruficorne*.

Water temperatures were favourable throughout the year for black fly life history development, although the marked cooling during autumn and winter is likely to lead to reduced numbers of generations over this period, and favour larger larvae that develop into more fecund adults (de Moor 1989). This is particularly so for the Prieska and Douglas sites, which were slightly cooler than the downstream sites.

Turbidity was a key driver in triggering ecosystem switching between dominance of major pest black fly species, and *S. adersi*/*S. nigritarse* co-occurring with benthic algae. Changes in turbidity caused switches in black fly species composition and abundance. A combination of reduced flows, increased water clarity and more alkaline water coincides with prevalence of benthic algae on rocks, and favours the “standard complex” fan structure blackly species, while the opposite of these variables favours conditions that increase the likelihood of major pest black fly outbreaks. The probability models indicate that under high turbidity conditions, *S. chatteri* is likely to dominate over *S. damnosum*. By contrast, the slope of the probability curves for both the benthic algae and *S. nigritarse* indicate slower responses to turbidity changes, compared to *S. chatteri*. Finally, the “weak complex” fan type species of *S. impukane* and *S. ruficorne* indicated a particular requirement for very clear water.

The negative correlation between the abundance of *S. chatteri* and *S. damnosum* showed that only one of these species dominates at any one time, with the other species largely excluded. With both species having similar hydraulic habitat preferences and labral fan structures (Palmer and Craig 2000), species dominance at any one site is likely to be governed by random and opportunistic colonisation; whichever species establishes first determines dominance. Changes in turbidity cause switches in black fly species populations and a concomitant increase in benthic algae, also noted in the Vaal River by de Moor (1994). Our models support the hypothesis that under natural conditions, flow variability in the Orange River would have driven a cycle of disturbance and opportunistic colonization,

whereas after impoundment the river had prolonged periods of lower flows and clearer water, alternating with prolonged periods of higher flows and elevated turbidity, and that these changes are the primary drivers that switch black fly species composition between the “strong porous” versus “standard” fan type black fly species. Also under lower flows, benthic algae and hydropsychids co-occur under clear conditions, where hydropsychid predation of black fly would be density-independent. Such patch dynamics (*sensu* Pickett and White 1985) would have been driven by biotic and physical factors, with temporal cycles being the most important for partitioning aquatic invertebrate cycles of dominance (Bogan and Lytle 2007). Just as in South Africa’s Great Fish River where a naturally non-perennial river system would have reset pest black fly populations annually (Rivers-Moore et al. 2007), it is most likely that the larger and perennial Orange River pest black fly populations would have been constantly reset before reaching pestilential numbers through regular and unpredictable periods of lower flows, when benthic algae would dominate. Regulated post-impoundment flows have guaranteed prolonged periods of dominance by the pest complex of *S. chutteri* or *S. damnosum*, necessitating an ongoing control programme. Under such conditions, mitigating factors such as predation also become miniscule, with water clarity likely to be a compounding constraint to hydropsychid predation on *S. chutteri* and *S. damnosum*, in addition to numbers of hydropsychidae considerably reducing for flow velocities > 1 m/s (Rivers-Moore et al. 2007). We conclude that black fly species composition is dynamic and reflects changes in flow and turbidity levels. Flow regulation has resulted in a trade-off between improved water supply and ecological costs resulting from reduced ecosystem variability, with what has been gained in terms of flow assurance and increased reticulation and water supply has come at the cost of reduced system resets and patch dynamics.

Acknowledgements

We thank the Water Research Commission (K5/2459: Dr Brilliant Petja) and Red Meat R&D SA for funding and supporting this research; Ms. Nthabiseng Kgotla and Ms. Mulalo Matodzi (Department of Agriculture, Forestry and Fisheries – Upington Office) for their support during field visits; Mr Sashin Naidoo and Ms. Esther Ndou for assistance with field work and sample processing. Reviewers are sincerely thanked for their comments.

References

- Bogan, M.T., and Lytle, D.A. 2007. Seasonal flow variation allows “time-sharing” by disparate aquatic insect communities. *Freshw. Biol.* **52**: 290-304.
- Brittain, J. 1991. Effect of temperature on egg development in the Australian stonefly genus *Austrocercella Illies* (Plecoptera: Notonemouridae). *Aust. J. Mar. Freshw. Res.* **42**: 107-114.
- Carlsson, G. 1967. Environmental factors influencing black fly populations. *Bulletin of the World Health Organization*, **37**: 139.
- de Moor, F.C. 1982. Determination of the number of instars and size variation in the larvae and pupae of *Simulium chutteri* Lewis 1965 (Diptera: Simuliidae) and some possible bionomical implications. *Can. J. Zool.* **60**: 1374-1382.
- de Moor, F.C. 1989. Alternative life-history styles in Simuliidae (Insecta, Diptera). *In Alternative Life-History Styles of Animals. Edited by M.N. Bruton.* Springer, Netherlands. pp. 293-316.
- de Moor, F.C. 1994. Aspects of the life history of *Simulium chutteri* and *S. bovis* (Diptera: Simuliidae) in relation to changing environmental conditions in South African rivers. *Verh. Int. Ver. Theor. Angew. Limnol.* **25**: 1817-1821.
- de Moor, F.C. 2003. Simuliidae. *In Guides to the freshwater invertebrates of southern Africa. Edited by J.A. Day, A.D. Harrison and I.J. de Moor.* Water Research Commission, Pretoria, South Africa. Vol. 9. pp. 75-109.
- Fredeen, F.J.H. 1977. Some recent changes in black fly populations in the Saskatchewan river system in western Canada coinciding with the development of reservoirs. *Can. Water Resour. J.* **2**: 90-101.
- Holling, C.S. 1973. Resilience and stability of ecological systems. *Annu. Rev. Ecol. Evol. Syst.* **4**: 1-24.
- Kilroy, C., and Biggs, B.J.F. 2002. Use of the SHMAK clarity tube for measuring water clarity: Comparison with the black disk method. *N.Z. J. Mar. Freshw.* **36**: 519-527.
- McCune, B., and Mefford, M.J. 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6.16 MjM Software, Gleneden Beach, Oregon, U.S.A.

- Mullins, W. 2007. Chapter 7 Cost-Benefit Analysis. *In* Guidelines for integrated control of pest blackflies along the Orange River by R.W. Palmer, N.A. Rivers-Moore, W. Mullins, V. McPherson, and L. Hattingh. WRC Report No. 1558/1/07, Water Research Commission, Pretoria, South Africa.
- Palmer, R.W. 1994. A rapid method of estimating the abundance of immature blackflies (Diptera: Simuliidae). Onderstepoort J. Vet. Res. **61**: 117-126.
- Palmer, R.W. 1997. Principles of integrated control of blackflies (Diptera: Simuliidae) in South Africa. WRC Report No. 650/1/97, Water Research Commission, Pretoria, South Africa.
- Palmer, R.W., and Craig, D.A. 2000. An ecological classification of primary labral fans of filter-feeding black fly (Diptera: Simuliidae) larvae. Can. J. Zool. **78**: 199-218.
- Palmer, R.W., Rivers-Moore, N.A., Mullins, W., McPherson, V., and Hattingh, L. 2007. Guidelines for integrated control of pest blackflies along the Orange River. WRC Report No. 1558/1/07, Water Research Commission, Pretoria, South Africa.
- Pickett, S.T.A. and White, P.S. 1985. The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, Orlando, Florida.
- R Development Core Team 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0, URL <http://www.R-project.org>
- Rivers-Moore, N.A., Dallas, H.F., and Morris, C. 2013. Towards setting environmental water temperature guidelines: A South African example. J. Environ. Manage. **82**:39-50.
- Rivers-Moore, N.A., Palmer, R.W., and Dallas, H.F. 2014. Assessing the relative culpability of *Simulium* (Diptera: Simuliidae) species in recent black fly outbreaks along the middle Orange River, South Africa. Can. J. Zool. **92**: 505-513.
- Rivers-Moore, N.A., de Moor, F.C., Morris, C., and O'Keeffe, J. 2007. Effect of flow variability modification and hydraulics on invertebrate communities in the Great Fish River (Eastern Cape province, South Africa), with particular reference to critical hydraulic thresholds limiting larval densities of *Simulium chatteri* Lewis (Diptera, Simuliidae). River Res. Appl. **23**: 201-222.
- Rodionov, S. 2006. Sequential regime shift detection v3.2. Pacific Marine Environmental Laboratory, NOAA/ PMEL, Seattle.

- Scheffer, M., Carpenter, S., Foley, J.A., Folke, C. And Walker, B. 2001. Catastrophic shifts in ecosystems. *Nature* **413**: 591-596.
- Schooler, S.S., Salau B., Julien, M.H. and Ives, A.R. 2011. Alternative stable states explain unpredictable biological control of *Salvinia molesta* in Kakadu. *Nature* **470**: 86-89.
- Skelton, P.H. 1986. Distribution patterns and biogeography of non-tropical southern African freshwater fishes. *In* Palaeoecology of Africa and the Surrounding Islands. *Edited by* E.M. Van Zinderen Bakker (Sr.), J.A. Coetzee and L. Scott. AA Balkema, Rotterdam. Proceedings of the VIIth Bienn. Conf. of the Southern African Society for Quaternary Research, University of Stellenbosch. Vol. 17. p. 211-230.
- Townsend, C.R. 1989. The patch dynamics concept of stream community ecology. *J. N. Am. Benthol. Soc.* **8**: 36-50.
- van Vuuren, L. 2012. In the Footsteps of Giants – Exploring the history of South Africa's large Dams. WRC Report No. SP31/12, Water Research Commission, Pretoria. ISBN 978-1-4312-0212-6.

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Table 1 List of study sites, showing site numbers and names in this study. Sites as a function of downstream distance (DD) from van der Kloof Dam, and elevation are reflected. Sites 8a-b were agricultural return flow channels, while site 9a was a small side channel.

Site	Latitude	Longitude	DD (km)	Elev. (m amsl)
1	-29.16194	23.69623	174.6	993
2	-29.65553	22.74592	355.1	926
3	-28.43743	21.40092	621.6	800
4	-28.45798	21.26165	636.5	785
5	-28.45262	21.25943	636.5	785
6	-28.60385	21.14277	660.3	778
7	-28.46768	21.10197	666.5	765
8	-28.68780	21.06878	671.8	746
8a	-28.68780	21.06878	671.8	746
8b	-28.68780	21.06878	671.8	746
9	-28.69490	21.01452	679.3	726
9a	-28.69490	21.01438	679.3	726
10	-28.72913	20.98595	683.8	724
11	-28.51377	20.18694	785.0	439

Table 2 Eigenvalues for seasonal water quality variables associated with the 14 black fly study sites on the middle and lower Orange River, with presence/ absence of benthic algae as a categorical value

	PC Axis 1	PC Axis 2
Cumulative % variance	45.23	79.04
Variables	Eigenvalues	
pH	0.236	-0.676
Turbidity	-0.673	-0.193
Conductivity	-0.611	-0.384
Benthic algae	0.344	-0.599

Draft

Table 3 Larval numbers for seven species of black fly sampled from 14 sites for the Orange River, South Africa, where Sade = *Simulium adersi*; Schu = *S. chutteri*; Sdam = *S. damnosum*; Smcm = *S. mcmaahoni*; Snig = *S. nigritarse*; S. ruf = *S. ruficorne*. NS indicates not sampled.

Date	Species	Sites													
		1	2	3	4	5	6	7	8	8b	8a	9	9a	10	11
Nov 2015	Sade	NS	NS												NS
	Schu	NS	NS	528	1104	95	124	485	50			29		320	NS
	Sdam	NS	NS	20	87	6	381	305	113			42		27	NS
	Snig	NS	NS								14				NS
March 2016	Sade			14	24	77	2	3	68			11	6	14	8
	Schu	3	3420	68	73	86	6	62	16			46		47	11
	Sdam			48	10	1	187	587	30			113		7	
	Snig			8			20	21	20		8	21		6	
	Sruf										1				
July 2016	Sade		5	390	225	680	132	122	82			86		7	43
	Schu	94	1853	125	310	88	56	119	41			66		11	85
	Sdam			3440	1128	883	266	678				1570		889	368
	Simp									46					
	Snig				55	46	13	18	72	3				40	10
Dec 2016	Sade	1077		3	205	695	98	1077	197					61	2
	Schu	1115	801	68	305	63	2	7				78		34	28
	Sdam	14		1348	2173	98	534	14	826			477		107	8
	Smcm	2						2						4	
	Snig										8				
	Sruf									2	3				

Table 4 Logistic regression model coefficients describing interactions between black fly and turbidity (Schu = *S. chutteri*; Sdam = *S. damnosum*; Snig = *S. nigriforce*; Simp-ruf = *S. impukane* and *S. ruficorne* labral fan complex), and benthic algae versus turbidity and black fly density scores (** $p < 0.001$; * $p < 0.01$; $p < 0.05$; $p < 0.1$; 46 d.f.)

	Schu	Sdam	Snig	Simp-ruf	Algae
Constant	9.12±2.69***	3.16±0.98**	-2.05±0.85*	-13.35±5.51*	4.06±1.63*
θ_{clarity}	-0.12±0.04***	-0.05±0.02**	0.03±0.02`	0.155±0.06*	-0.06±0.03`
θ_{density}					-0.27±0.14`

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Figure Captions

Figure 1 Conceptual grouping of Orange River black fly species by fan type, based on seston concentration and velocity preferences (Palmer and Craig 2000). The two most abundant pest species fall within the dotted line ellipse.

Figure 2 Principal components analysis plot showing distribution of sampling sites of four sampling seasons, based on water quality. Sites were defined in terms of algal presence or absence, with axes 1 and 2 accounting for 45 and 34% of variability respectively

Figure 3 Mean daily water temperatures at Prieska over one year, showing thermal seasons defined as being where statistically significant changes in mean daily water temperatures occur. The monthly frequency of occasions when flow and turbidity conditions (TSS) were likely to favour benthic algae has been superimposed.

Figure 4 Bray-Curtis ordination of sites surveyed, based on simuliid species data (percent contribution of each species' relative abundance to total number per sample). Ellipses represent site groupings based on cluster analysis; season codes: 1 = late spring; 2 = late summer; 3 = winter; 4 = early summer.

Figure 5 Negative correlation between abundance of *Simulium chatteri* (Schu) and *S. damnosum* (Sdam), with changes in seasonal dominance between both species based on percentage contribution (inset)

Figure 6 Potential numbers of black fly and hydrosychid caddisfly larvae as a function of density classes for a habitat area of 90 000 cm², and associated predator: prey ratios

Figure 7 Probability of occurrence of seven black fly species as a function of water clarity (cm), and benthic algae

Figure 8 Frequency of occasions when benthic algae was likely to dominate under lower-flow, higher clarity for the Orange River from 1942-1977 (pre-impoundment conditions) and 1978-August 2016 (post-impoundment conditions)

Figure 1

Figure 2 Principal components analysis plot showing distribution of sampling sites of four sampling seasons, based on water quality. Sites were defined in terms of algal presence or absence, with axes 1 and 2 accounting for 45 and 34% of variability respectively

176x173mm (96 x 96 DPI)

Figure 3

Figure 4 Bray-Curtis ordination of sites surveyed, based on simuliid species data (percent contribution of each species' relative abundance to total number per sample). Ellipses represent site groupings based on cluster analysis; season codes: 1 = late spring; 2 = late summer; 3 = winter; 4 = early summer.

254x190mm (96 x 96 DPI)

Figure 5

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Figure 6

Figure 7

Figure 8