Refining prediction accuracy for pest blackfly outbreaks using Bayesian networks, Orange River, Northern Cape, South Africa

by

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ABSTRACT

Construction of dams, impoundments and Inter-Basin Transfer schemes (IBTs) along the Orange River are aimed to provide useable water for multiple sectors. However, operation of these water schemes had led to changes in flow regimes, seston concentrations and water temperatures, which has led to an escalation of pest blackfly (*S.chutteri*) outbreaks along the lower to middle reaches of the Orange River. Pest blackfly bite livestock, poultry and humans as they require a blood meal to complete ovarian development. During outbreak periods, livestock farming and the grape industries are affected negatively by pest blackflies along the Orange River. The blackfly control programme has been operating for over twenty years, and aims to control blackfly outbreaks by applying larvicides along the Orange River. Although this programme is in place, periodic outbreaks occur and losses in livestock and productivity can amount to an estimated R300 million during an outbreak (2013). Therefore, other methods should need to be integrated with this programme to achieve blackfly control. Predictive modelling was identified as a method to assist the blackfly problem. Being able to predict when, where and the severity of an outbreak, will assist management in control planning. Bayesian network (Bn) models were identified as a suitable predictive model, as multiple variables can be used in understanding the cause and effects of a response variable. The aim of the research was to refine prediction accuracy of blackfly outbreaks along the middle to lower reaches of the Orange River, using Bns. Fourteen sites were sampled along the Orange River, for which abiotic and biotic data were collected during four sampling periods. These data were used in assisting quantitative components of the Bns, whilst the qualitative components were based of previous Bns with additions on new nodes that were identified as affecting blackfly outbreaks. Water temperature data showed that sites were split into two distinct groupings, for which Bns were constructed. These were termed the upper and lower stream models. The upper stream model had the higher outbreak probabilities, whilst it was predicted for both models that summer would be the season most likely for an outbreak to occur. The species most likely to cause an outbreak was identified to be either *S.chutteri* or *S.damnosum*, with switching in dominance throughout sampling periods potentially due to switching in seston concentrations. Future outbreak probabilities based on scenarios of increased discharge and water temperatures indicate that the blackfly problem is likely to worsen, with increases in discharge resulting in greater habitat availability for pest species and increases in water temperature resulting in shorter life cycles and more rapid reproduction. The Bns constructed show promise in assisting management as blackfly outbreak probabilities were refined on a spatial and temporal scale along the middle to lower reaches of the Orange River.

Declaration

I ......Sashin Naidoo.. declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

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(vii) This research shared a common dataset, which were used for this research, the work of MSc candidate Esther Ndou and for the WRC project K5/2459 final report.

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Chapter One

Introduction

1.1. Introduction

While redistribution of water between catchments through dams, impoundments and inter-basin transfer schemes (IBTs) are beneficial to agricultural, industrial and human use, construction of water schemes has resulted in permanent changes in seasonality and environmental variables within river systems (Snaddon *et al*., 1998; Rivers-Moore *et al*., 2008a; Dallas and Rivers-Moore, 2012). Flow volumes and water temperatures play a pivotal role within river systems and shifts in these variables can have permanent effects on the system (Rivers-Moore *et al*., 2013a). Dallas and Rivers-Moore (2012) note that dams, impoundments and IBTs modify the water temperature and flow volumes of a system post-construction (Fredeen, 1977; Fredeen, 1985; Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2008b; Rivers-Moore *et al*., 2014). Such changes were observed in the Orange River after the Gariep and Vanderkloof dams were constructed in the 1970s, and in the Great Fish River after the completion of the IBT from the Orange River (Myburgh and Nevill, 2003; Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2008b; Rivers-Moore *et al*., 2013a; Rivers-Moore *et al*., 2014). These changes have negative impacts on the ecological functioning which can result in health and economic risks within that environment (Rijsberman, 2006; Rivers-Moore *et al*., 2008a).

Changes in environmental variables, particularly water quality, after water schemes are operational cause human health concerns. Furthermore, the development of water resource management strategies and transfer schemes have introduced new diseases or intensify old diseases in areas in which they have been constructed (Steinmann *et al*., 2006). Even though operation of dams and impoundments are needed to meet the food and energy requirements of countries with high populations, millions of people are at risk due to diseases such as schistosomiasis (Steinmann *et al*., 2006). This is a parasitic disease which has been contracted worldwide by approximately 100 million people that live near large dams and irrigation schemes. With 3000 dams being built worldwide in the 1990s alone, such diseases are widespread and are likely to increase (Steinmann *et al*., 2006).

Although there are direct economic benefits attributed to dams, impoundments and IBTs, there are indirect economic impacts that are attributed to their construction. Seasonal variation of flow is reduced post-construction, and this change in variability affects the ecology of the area (Rivers-Moore *et al*., 2008b; Rivers-Moore *et al*., 2014). A change ecology has an indirect impact on the economy of the area, as an introduction of pest species into the area can have an impact on the agricultural and tourism sectors (Rivers-Moore *et al*., 2014). Such an example is the increase of blackfly (Diptera: Simuliidae) outbreaks due to favourable development conditions post-construction (Myburgh and Nevill, 2003). Some species are considered significant pest species within major river systems in countries such as South Africa, Canada, Serbia and Ghana (de Moor, 2003; Rivers-Moore *et al.,* 2014).

1.2. Blackfly (Diptera: Simuliidae)

Jones and Kitching (1981, cited in Worner and Gevrey, 2006, pp 859), define a pest as “an organism that damages crops, destroys products, transmits or causes disease, is annoying or in other ways conflict with human needs or interest”. A few blackfly species meet these criteria, and therefore can be classified as ‘pests’. Blackflies belong to the Simuliid family of the Diptera (flies) and are ubiquitous in freshwater systems worldwide (Zhang *et al*,.1998; Lautenschläger and Kiel, 2005). Distribution of different species of blackflies are generally habitat-specific as some are found in slow flowing systems whilst others inhabit large rivers with swift and turbulent water (Palmer and Craig, 2000). Changes in flow velocities, turbidity and water temperature due to water transfer schemes can result in a switching of species from naturally occurring species to invasive pest species (Fredeen, 1985), and abundance of pest species can increase dramatically, leading to severe economic losses and health concerns depending on the blackfly species.

Blackflies are carriers of diseases and countries within western Africa suffer from transmitted diseases such as *Onchocerciasis* commonly known as ‘River Blindness’ (Lamberton *et al.,* 2014). To-date, South Africa have not had any reported cases of *Onchocerciasis*, however, outbreaks have high economic impacts on the country. While there are 39 species of blackfly that are currently identified in South Africa, the main pest species occurring within the country is *Simulium chutteri* (Palmer 1997; Myburgh and Nevill, 2003). This species is endemic to southern Africa and is found within large river systems under favourable conditions (Myburgh and Nevill, 2003). Within South Africa, rivers including the Orange, Great Fish and Vaal experience periodic outbreaks from such species and are classified as significant pest species (Myburgh and Nevill, 2003; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2013a). Other species that may be problematic within the country, particularly along the Orange River, are *S. damnosum*, *S.adersi, S. nigritarse* and *S. impukane* (de Moor, 2003).

Understanding the conditions that determine blackfly species distributions and densities are important, as control measures by management and programmes are based on sound ecological knowledge (Carlsson, 1967). The distribution and abundance of blackfly species are associated with environmental factors and therefore their presence is often predictable based on the conditions found within the river system (Hamada *et al*., 2002). Labral fan morphology is linked to habitat preference for blackfly species and factors such as stream size, flow and seston concentration are identified to be significant determinants in the labral fan morphology of blackfly species (Palmer and Craig, 2000; Hamada et al., 2002). *Simulium chutteri* generally prefers habitats with high velocities (flows in excess of 1 $m.s^{-1}$) and high seston concentration due to their labral fan structures enabling them to feed efficiently at high velocities (Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2014). Labral fan structures of other blackfly species such as *S.impukane* and *S.nigritarse* are adapted to habitats that have lower flow velocities (flows less than 1 $m.s^{-1}$) and lower seston concentrations (Palmer and Craig, 2000; Rivers-Moore *et al*., 2014). This implies that labral fan structure is a determinant for where certain species are more likely to occur. Species such as *S.chutteri* and *S.damnosum* would be most likely found in riffles and rapids where there are high flow velocities and seston concentrations whereas *S.nigritarse* and *S.impukane* for example are most likely to be found in small clear streams, which are characteristic of side streams (Rivers-Moore *et al*., 2014). For control purposes, knowing where pest species are found and knowing when during the year they are most likely to be at their most abundant is important for control measures. The greatest *S.chutteri* larvae and pupae populations are generally expected during late winter to early spring based on their life cycle and abiotic conditions during these months. Therefore, understanding the morphology and life cycle of blackfly species is fundamental for effective control measures (Hamada *et al*., 2002).

1.3. Blackfly Control Programmes

In South Africa, the blackfly Control Programme for the Orange River has been in place for approximately twenty years, with mixed success (Table 1.1). Potentially this should have resulted in successful control of blackfly outbreaks. However, this is not the case, as periodic outbreaks of blackfly continue to occur, and the monitoring dataset is irregular and seldom evaluated. In addition, conditions along the Orange River have changed with the completion of Phases 1a, b of the Lesotho Highlands Water Programme, resulting in changes in water quality, flow patterns and therefore blackfly species (Rivers-Moore *et al*. 2014; Rivers-Moore and Palmer, 2017). Therefore, there is a need to re-consider previous research within a new predictive framework. Pilot studies regarding blackfly outbreaks (Rivers-Moore *et al*., 2007) have been conducted within the Great Fish River, however, due to the greater economic importance that the Orange River has, emphasis on outbreak prevention needs to be prioritised within this system.

Table 1.1: Matrix of successes and failures of the Orange River blackfly Control Programme (Rivers-Moore and Palmer, 2017).

Success (Y)/ Challenge (X)	Aspect	Comment
Y	Monitoring system	Ten-point scoring system developed by Palmer (1994) is scientifically robust and simple to use.
X	Monitoring Programme	Some a degree of success, but has gaps in the dataset, and has not been audited.
X	Larvicide application	The method has been successful, although larvicide resistance has developed.
X	Flow manipulation	In theory a possibility, but in practice difficult to implement due to time lags in downstream flow reductions, and because of competing needs from Eskom for higher winter flows for power generation.
X	Integrated approach	This has been the approach recommended by blackfly research experts, but to-date remains difficult to implement.
X	Stakeholder involvement	Palmer *et al*. (2007) suggested a management structure to manage the blackfly problem, but to-date this has not been realised.
Y	Probabilistic integrated approach	Has a good chance of managing the problem, as it allows for competing variables to be interactively assessed.

1.4. Impacts of blackfly outbreaks along the Orange River

In situations where the Orange River blackfly Control Programme is not operating effectively, blackfly outbreaks can result in agricultural losses of approximately R300 million per annum to the local economy (Rivers-Moore *et al*. 2014). Such outbreaks occur periodically, with the most recent in 2011 (Rivers-Moore *et al*., 2014) and a previous outbreak reported in 2000-2001 (Palmer *et al*., 2007). Economic losses occur along a length of some 1200 km along the middle and lower Orange River between Hopetown and Sendelingsdrif. Typically, the blackfly species causing the problem is *S. chutteri*, with more than 250 breeding sites (riffles) identified along the affected river sections, although other species including *S.damnosum, S.nigritarse* and *S. impukane* may cause periodic outbreaks (de Moor, 2003). The outbreaks occur despite an integrated Control Programme framework described by Palmer (1997), using a rapid ten-point qualitative scoring system to monitor weekly larval and pupal densities at up to 14 sites along the affected river length (Palmer, 1994). The basis for this programme has been to target the aquatic larval stages of primarily *S. chutteri* using helicopter-based application of two larvicides, and ideally in response to anticipated outbreaks from the monitoring programme. Due to the lack of understanding of environmental factors such as flow velocities, seston centration and water temperature and their effects on blackfly distribution and abundance, it is difficult for such monitoring programmes to anticipate outbreaks. Knowledge of what species are most likely to be found where under certain conditions, and the abundance of species needs to be known for effective control. However, this information is difficult to manually gather across a large system such as the Orange River. Predictive modelling of the system is thus an ideal way to overcome this limitation.

1.5. Predictive Modelling

Predictive modelling can be applied for studies with large datasets, which makes it a desirable tool (Zukerman and Albrecht, 2001; Dickey, 2012). Predictive models involve determining mathematical relationships between 'response' variables and 'independent' variables with the intention of predicting future values of the 'response' variable (Dickey, 2012). Modelling has been made possible due to advancements in machine learning capabilities (Zukerman and Albrecht, 2001). Some of the models used for predictive modelling include: linear models, Markov models and Bayesian network models (Bns) (Zukerman and Albrecht, 2001). The Orange River system has multiple variables which impacts blackfly outbreaks, which is the 'response' variable. Predicting when and why blackfly outbreaks are likely to occur could benefit management for control measures. A model that considers multiple variables is needed in such systems for accurate predictions. Models such as the linear model and the Markov models characteristically make predictions regarding a single variable and are not suitable for predictions of multiple variables (Zukerman and Albrecht, 2001). However, Bns have proven to be useful to predict the probability of multiple variables on a response variable (Zukerman and Albrecht, 2001; Rivers-Moore *et al*., 2014). Bns do not rely on large sets of empirical data such as other traditional statistical models, and can be used to classify or predict events or states with uncertain or limited data (Chen and Pollino, 2012). Furthermore, they are useful as they can handle missing data and allow the combination of various types of data including; quantitative and qualitative data, and expert opinion (Uusitalo, 2007; Henriksen and Barlebo, 2008; Chen and Pollino, 2012). Bns could therefore be advantageous in systems such as the Orange River where multiple variables affect the ‘response’ variable. With there being gaps in the monitoring dataset for the blackfly monitoring programme, Bns could be applied as they adapt to dealing with such data without hindering the validity of the model which is why Bns was the selected model for this research.

1.6. Research aim and objectives

Bayesian networks can be used to predict when, where and what type of blackfly population outbreaks are likely to occur along the middle to lower Orange River.

1) Develop structural components of the Bayesian networks (Bns).

2) Identify spatial and temporal resolution for the Bns.

3) Populate the Bns ‘parent’ and ‘child’ nodes by calculating probabilities from time series and field data.

4) Generate blackfly outbreak probabilities under current and potential conditions and verify predicted outbreak probabilities to known outbreak probabilities.

1.7. Structure of thesis

The thesis is structured in six chapters, including this introductory first chapter. A review of literature relevant to the research is presented in chapter two, which includes blackfly ecology and causes of outbreaks, management along the Orange River, environmental modelling and Bayesian networks (Bns). Chapter three consists of the methods section which includes sampling sites and descriptions, abiotic and biotic data collection and the process and data used for the construction of the Bayesian networks (Bns). The results were analysed and presented in chapter four which include abiotic and biotic data, thresholds and critical values determined for blackfly species and nodes with the Bns and outbreak probabilities generated for each spatial Bn under current conditions and future scenarios. Chapter five and six were the discussion and conclusion section of the thesis, included in these two chapters are discussions on abiotic and biotic variations along different regions of the Orange River which impacted the spatial resolutions of the Bns and conclusions on outbreak probabilities generated from the Bns.

Chapter Two

Literature Review

2.1. Introduction

Blackfly outbreak prediction in South Africa is the focus of this research, with *Simulium chutteri* being the main species reviewed in the literature. *S.chutteri* is viewed as the main problematic species in South Africa, therefore life histories were prioritised and reviewed. However, other blackfly species were reviewed in terms of impacts in other countries. The control programme has been initiated along the Orange River to prevent blackfly outbreaks as they cause severe economic losses to surrounding areas (Rivers-Moore *et al*., 2014). Control measures discussed are; larvicide applications, flow manipulation and the potential of probabilistic modelling as an option for control managements. Bayesian network models (Bns) is a type of probabilistic model, and this research identifies this model as an option for predicting outbreak probabilities of blackfly species along the Orange River (Rivers-Moore *et al*., 2014). The advantages of Bns and how multiple variables can be used to predict the probabilities of a 'response' variable are reviewed. Discharge, seston concentration and water temperature are all variables that influence blackfly abundance and distribution and therefore can be used to predict this probability of blackfly outbreaks. Being able to predict outbreak probabilities of blackfly species will benefit management and stakeholders along affected reaches of the Orange River.

2.2. Blackflies (Simuliidae: Diptera)

2.2.1. Blackfly Ecology

Simuliids are flies with a global distribution (de Moor, 2003). Their larvae are considered important components of the benthic fauna in flowing waters and are often used as indicators of environmental degradation and restoration (Kazanci, 2006). Blackflies, gnats and midges belong to the Simuliidae family with some of these species having significant economic and medical impacts (de Moor, 2003). Blackflies are particularly dominant, as in ideal conditions, 95% of the invertebrates collected from substratum within rapids, such as stones and trailing vegetation, can consist of blackfly larvae and pupae (Zhang *et al.,*1998; Kiel, 2001; de Moor, 2003; Lautenschläger and Kiel, 2005). There are approximately sixty-five blackfly species described within southern Africa (de Moor, 2003). These organisms have four main stages of their life-cycle: egg, larval, pupal and adult. The larval stages are associated with a varying number of instars which are species dependent, and can be found in a range of habitats from slow flowing streams to large fast flowing rivers (Palmer and Craig, 2000), however, this is species-specific. Flow velocity, seston concentration and water temperatures have been identified as key components affecting blackfly abundance and species, whilst anthropogenic impacts such as disturbances to rivers riparian vegetation removal can affect species abundance and composition (Lautenschläger and Kiel, 2005). The abundance in blackfly populations are due to the larvae being efficient colonizers of suitable habitats, and in habitats of fast flowing waters blackfly larvae are often the dominant suspension feeders (Zhang *et al.,*1998). Their streamline body shapes and their body positioning in relation to water currents make them efficient filter feeders (Kriel, 2001). Blackflies play a pivotal role in the food web within water bodies. They serve as an important source of food for predators (Carlsson, 1967; Palmer and Palmer, 1995), and to downstream suspension feeders as they release undigested particles (Lautenschläger and Kiel, 2005). There are species of blackfly adults that are considered pests, as transmission of vectors and diseases to humans and animals are associated with female blackflies (de Moor, 1989; de Moor, 2003; Myburgh and Nevill, 2003).

2.2.2. Blackfly lifecycle

Blackfly species undergo rapid larval development and females usually require a blood meal to complete ovarian development (de Moor, 1989; Myburgh and Nevill, 2003). Therefore, the females bite and feed on livestock, poultry and humans (de Moor, 2003). Species found within colder climates tend to develop at slower rates as the cold conditions only allows for the completion of one generation per annum, and adult emergence can be predicted at certain times of the year (de Moor, 1989). Warmer regions allow for the completion of more than one generation per annum (de Moor, 1989). The reason for this is assumed to be that colder climates force the species to spend most of their reproductive efforts towards achieving the highest possible survival rate of their offspring, whereas in warmer climates the species focus their effort on rapid reproduction of offspring and less effort on the timing or survival of the eggs (de Moor, 1989).

In South Africa, *Simulium chutteri* breed throughout the year and do not hibernate during winter, which is one of the reasons for its dominance of the system (de Moor, 1982; de Moor, 1989). The life history of *S.chutteri* suggests that the life stages varies during different times of the year. The life span of these species from egg to adult stage is between 12-24 days depending on seasonal and abiotic variables such as water temperature (de Moor, 1989). During winter months, larvae that experience sudden drops in temperature are often induced to a state of quiescence or growth retardation (de Moor, 1989; Rivers-Moore *et al*., 2014). However, eggs that hatch during or after this sudden drop in temperature continue their development, at a slower rate, as they have become acclimatised to the conditions (de Moor, 1982; de Moor, 1989). The acclimatized larvae are generally the larger larvae due to development at a more efficient thermal equilibrium (de Moor, 1982). Therefore, there are two different generations of *S.chutteri* within the winter months; the acclimatised generation and the generation that is in a state of quiescence which carries on development when water temperature increases (de Moor, 1989). This provides higher fecundity amongst the females which leads to potential spring outbreaks (Rivers-Moore *et al*., 2014). Colder conditions result in larger eggs and less offspring, whilst warmer conditions result in smaller but more eggs and offspring (de Moor, 1982; de Moor, 1989). It is predicted that climate change in naturally cold environments could cause emergence of offspring at an earlier than anticipated time (Carlsson, 1967). Therefore, climate change could exasperate emergence times with increasing water temperatures. Potentially the winter months may not have cold enough water temperatures to influence larvae to be induced to in a state of quiescence. This could lead to multiple generations per annum, however, due to their being no water temperatures low enough to induce quiescence, spring outbreaks may not occur as there would not be two different generations emerging at the same time (de Moor, 1982).

Peak blackfly larvae and pupae densities are expected to be found in the winter due to there being more than one generation of blackfly developing during this period (de Moor, 1982; de Moor, 1989). Beside water temperature affecting development and during winter, larval and pupal development do not face many threats during the winter to early spring period (de Moor, 1989). Natural blackfly predators such as larval hydropsychids are not abundant during this period and therefore blackfly larvae and pupae do not face threats from biological factors (de Moor, 1989). Historically, low and variable flows during winter assisted in controlling blackfly populations along the Orange River, as low flows expose substrate to sunlight and desiccate blackfly larvae and pupae (Palmer, 1997). However, post construction, winter periods have had increase flows and decreased in flow variability which have encouraged blackfly populations (Palmer, 1997; Rivers-Moore *et al*., 2014). These factors contribute to the reason why spring is the most likely season in which outbreaks are expected, as adult emergence will be high during this period. Spring has favourable flow conditions with high flows experienced during this season, and suitable substrata available in the waters which leads to adult laying eggs during this period as abiotic conditions are ideal (Carlsson, 1967). The re-emergence of blackfly predators during this season may result in a control in blackfly populations which will limit the adult emergence and outbreak likelihood during summer. However, it is possible for there to be adult outbreaks and annoyance during summer as farmers along the Orange and Vaal Rivers suggest that the months of November, December and January are when they experience the greatest blackfly annoyances (de Beer and Green, 2012). Therefore, winter and spring will be the months were there will be the highest densities of blackfly pupae and larvae, but spring and summer will be the months where adult emergence and outbreak likelihoods will be highest. Therefore, for effective control along the Orange River, if the size of the population of *S.chutteri* larvae and pupae within the late winter to early spring period can be reduced, there is less chance for an outbreak that will be problematic for livestock and stakeholders (de Moor, 1982; Rivers-Moore *et al*., 2014).

2.2.3. Blackfly Outbreaks

Outbreaks of blackfly impact upon the economic, veterinary and medical sectors. Canada, Serbia, Ghana and South Africa are just a few of the countries that have been plagued by periodic blackfly outbreaks. Examples of this can be seen within the Saskatchewan River system in Canada, where economic losses were experienced in 1978 due to blackfly outbreaks *(S. luggeri and S. vittatum)* severely impacting livestock production (Fredeen, 1985; Rivers-Moore *et al*., 2014). Different blackfly species outbreaks in Serbia from 1958 to 1970 have heavily impacted livestock, poultry and humans (Ćupina *et al.,* 2014). *S. colombaschense* was responsible for losses in livestock productivity, *S. maculatum* resulted in a decrease in poultry production and *S. erythrocephalum* caused dermatological concerns for humans (Ćupina *et al.,* 2014). This is an indication that different blackfly species can lead to economic losses in different regions and sectors. Besides being an economical hindrance, blackfly have been responsible for transmitting diseases due to the blood feeding requirements within their life cycle (Myburgh and Nevill, 2003). The common disease transmitted to humans is *Onchocerciasis* which is known as 'River

Blindness', whilst some humans have allergic reactions to blackfly bites known as simuliotoxicosis or more commonly 'blackfly fever' (Myburgh and Nevill, 2003).

The severity of 'River Blindness' has led to many efforts and programmes being implemented to prevent this disease. Western Africa and South America have an estimated 20 million people infected and blinded due to this disease (Myburgh and Nevill, 2003). The filarial nematode worm *Onchocerca volvulus* is responsible for the disease hence the name *Onchocerciasis* (Myburgh and Nevill, 2003; de Moor, 2003; Lamberton *et al.,* 2014). Blackflies are carriers of this nematode worm which are transmitted to humans once they have been bitten (de Moor, 2003; Lamberton *et al.,* 2014). The most common blackfly species complex in countries such as Ghana that carries this nematode worm is *Simulium damnosum (S.damnosum)* (de Moor, 2003; Lamberton *et al.,* 2014). This species is common in South Africa, however, no cases of 'River Blindness' have been reported (de Moor, 2003). The main concern in terms of blackfly outbreaks within South Africa are towards livestock productivity and loss. Although primary diseases and viruses within animals have been attributed to blackfly bites, bites on body parts that are exposed, such as eyes and ears can lead to secondary infections from exposed wounds, which lead to the loss of ears and udders in livestock (de Moor, 1989; Palmer and Palmer, 1995; Palmer, 1997; Myburgh and Nevill, 2003). Economic losses resulting from these pest species have led to control programmes being initiated in South Africa.

Periodically, the middle to lower regions of the Orange River suffers significant economic losses attributed to blackfly (Diptera: Simulidae) outbreaks (Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2008b; Palmer and Rivers-Moore., 2008). Sectors particularly vulnerable to these outbreaks are the tourism and agricultural sectors, with losses in livestock, production and annoyance to labour severely affecting the sheep and grape industries (Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2014). Outbreaks along the Orange River can lead to losses more than US$30 million (R300 million) (Rivers-Moore *et al*., 2014), and this figure likely to rise due to inflation of meat prices.

The blackfly species which dominates the Orange River system is *S.chutteri*, which is endemic to South Africa (de Moor, 2003). *S.chutteri* shows characteristics that can be classified as a pest species, with other outbreaks recorded in the Vaal and Great Fish Rivers (Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2008b). However, the economic impact within these regions are not as severe as on the Orange River due to the magnitude and importance of this system to the country's economy. Although 'River Blindness' is not prevalent in South Africa, the species responsible for the transmitting this disease in western Africa, *S.damnosum*, are abundant in major South African river systems (de Moor, 2003). Therefore, even though blackfly outbreaks in South Africa are associated with economic losses and impact on livestock and poultry (Rivers-Moore *et al*., 2014), the health risks that blackflies could potentially inflict on humans should not be ignored.

2.3. Downstream effects of dams and impoundments

Due to South Africa's semi-arid characteristics and low annual mean precipitation, dams, impoundments and Inter-Basin Transfer schemes (IBTs) were constructed to alleviate water shortages and redistribute water to areas in which water is scarce (Snaddon *et al*., 1998). After the completion of the Gariep and Vanderkloof Dams in 1977, there has been an escalation in the blackfly problems along the Orange River (Myburgh and Nevill, 2003; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2013a). These dams have altered downstream flow regimes and decreased flow fluctuations and variability especially in the winter months which lead to favourable conditions for pest blackfly species (Rivers-Moore *et al*., 2014). Whilst IBTs are not responsible for the escalation of blackfly outbreaks along the Orange River, blackfly problems have occurred in the Great Fish River due to flow modifications caused by IBTs (Rivers-Moore *et al*., 2008a). Dams and IBTs are responsible for ecological change post-construction. Ecological change is attributed to alterations in flow, seston concentrations and water temperatures within river systems, and the risk of ecological change is dependent on the magnitude of the alteration (Rivers-Moore *et al*., 2013a). With the construction of dams and IBTs, a decrease in flow variability has led to a changed community structure and have a negative impact on species sensitive to certain environmental thresholds (Rivers-Moore *et al*., 2013a; Rivers-Moore *et al*., 2014). Other factors leading to suitable conditions for blackfly larvae are; less seasonal flow variability and increased suspended organic material which can be attributed to the development of dams, impoundments and IBTs (Myburgh and Nevill, 2003). Habitats in which blackflies are found can be species-specific (Palmer and Craig, 2000). Although there are many variables that can make a habitat species-specific and affect blackfly distribution and abundance, the main variables that will be the focus in the literature are flow velocity, seston concentration and water temperatures. This is due to the direct linkages these variables have on blackflies in terms of feeding efficiency or development.

2.3.1. Flow velocities

Blackfly species have varying flow velocity preferences. *S.chutteri* and *S.damnosum* prefer fast flowing waters in which velocities are greater than 1 $m.s^{-1}$, whilst *S.impukane and S.nigritarse* prefer slow flowing waters where the velocity is less than 0.5 $m.s^{-1}$ (Palmer and Craig, 2000; Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2014).

Dams, impoundments and IBTs provide a system with reassured flow volumes, which have clear benefits, however, this results in a reduction in flow variability and seasonality and this homogenisation of conditions are a major influence towards the increase in blackfly outbreaks (Rivers-Moore *et al*., 2013a). Homogenisation of environmental conditions within the Orange River allow for flow conditions that are ideal for the blackfly species to dominate as they are more stable and constantly above the critical velocities in which species such as *S. chutteri* prefer (Rivers-Moore *et al*., 2013a). This is due to the reduction in variability caused by natural seasonal shifts. According to Rivers-Moore *et al* (2008), the increase of high-velocity biotopes within the systems are favourable towards *S.chutteri* dominance and outbreaks. A change in flow and current velocity can lead to migrations where blackfly adults lay their eggs in habitats more suitable for growth and development (Carlsson, 1967). As a result, there are shifts in species composition and dominance. Species most suitable to the new flow regimes of the area increase exponentially in numbers and dominate the system, and previously dominant species which were adapted to previous flow regimes are not as abundant. The new species that are now dominant can have severe economic consequence on the system (Rivers-Moore *et al*., 2014). This was noted by Fredeen (1985), who observed the change in community composition of blackflies which was caused by the IBT construction along the Saskatchewan River system in Canada. The IBT negatively influenced the development of *Simulium articam* larvae and encouraged the development of *Simulium luggeri* and *Simulium vittatum*, due to changes in the conditions of environmental variables within the system (Fredeen, 1985). This was similarly the case in the Orange River, with changes in flow regimes resulting in previous dominant species *S.adersi* and *S.nigritarse* being now found in lesser abundance, whereas *S.chutteri* numbers have increased due to favourable flows (O'Keeffe and de Moor, 1988). It is clear that flow velocities impact blackfly distribution and abundance and a shift in flow velocities can lead to a shift in dominant blackfly species and abundance.

2.3.2. Seston concentration

With dams, impoundments and IBTs changing the seston concentration of rivers post construction, a switch in blackfly species dominance could occur (Fredeen, 1985). Organic carbon within running water is primarily found in suspended particles, which affects suspension feeders' life history and production dynamics (Zhang *et al.,* 1998). Particle concentration within the water has been identified as a major determinant, along with flow velocities, for blackfly larval distribution (Palmer and Craig, 2000; Rivers-Moore *et al*., 2014). Suspended particles are captured by blackfly larvae non-selectively with the aid of their labral fans (Zhang *et al.,* 1998). Blackfly species have different labral fan structures and therefore different preferences to flow velocities and seston concentration. Labral fan morphology of species found in fast flowing waters have small fans with many short and strong rays, whilst species in slow flowing waters have large fans with long and delicate rays (Palmer and Craig, 2000). Habitat preference could be determined by the labral fan structures of each species. Blackfly species can be grouped by labral fan structure according to Palmer and Craig (2000), with species being classified in complex groups. The strong porous complex group is for species adapted to high seston concentrations (and high flows) whereas the weak complex group is for species adapted to low seston concentrations (and low flows) (Palmer and Craig, 2000). *S.chutteri* for example is adapted to habitats that has high seston availability (>50 mg.ℓ^{-1}), whilst *S.impukane* is adapted to low seston habitats (<10 mg.ℓ^{-1}) (Palmer and Craig, 2000; Rivers-Moore *et al*., 2014). Therefore, seston concentrations of a system will influence the type and abundance of blackfly species found.

2.3.3. Water temperature

Construction of water transfer schemes, human induced activities, river regulations and climate change are some of the factors which modify water temperature (Dallas and Rivers-Moore., 2012). Macro-invertebrates have different thermal ranges and thresholds and changes in water temperatures could lead to different sensitivity levels amongst species (Rivers-Moore *et al*., 2014). Modifications can then lead to changes in macro-invertebrate life cycles and exasperate pest outbreaks within river systems (Rivers-Moore *et al*., 2013a; Rivers-Moore *et al.,* 2014). Seasonal changes in water temperature and food availability changes leads to variations larval and pupal sizes of blackfly (de Moor, 1982). Dallas and Rivers-Moore (2012), suggests that water temperature has impacts on organisms including; growth, emergence, fecundity and behavioural stresses with increased temperature including increased movement and a loss of grip that species have on a substrate. Water temperature can affect species at an individual to community level in terms of distribution and composition (Dallas and Rivers-Moore., 2012). Water temperature increases, decrease the aquatic stages of species life cycle, whilst there are decreases in larval instars in certain species due to temperature increases (de Moor, 1982). Lower water temperatures result in slower growth, which could result in larger larvae. Each blackfly species has different optimum temperatures in their larval and pupal stages, with the value for most species being greater than 12^{o} C (Carlsson, 1967). Increase in water temperatures results in an increase in development rate which may result in adult emergence all at a similar time (de Moor, 1982). The study conducted by de Moor (1982), indicates that temperature is likely to affect the growth rate of the pest species *S.chutteri*. Therefore, changing water temperatures along the Orange River due to construction of dams, impoundments and IBTs can have a serious impact on river systems due to exasperated pest outbreaks with water temperature impacting the size of larvae and the number of generations per annum. With the threat of climate change, increasing or fluctuations in water temperatures could increase the occurrences of blackfly outbreaks within South Africa.

2.4. Management of blackflies

2.4.1. Early larvicide applications

Although blackflies can be considered pest species, they serve as an important source of food for many aquatic predators (Carlsson, 1967; Palmer and Palmer, 1995). Therefore, eradication of this species should not be the goal but rather the objective should be to control population numbers so that they do not reach severe outbreak proportions. Blackfly control can be achieved with the following; flow manipulation, biological control and larvicide application. The blackfly control programme along the middle and lower Orange River is based on aerial applications of larvicides to control the pest species *S.chutteri*. South Africa's early form of control method for blackfly populations was DDT application to rivers (Myburgh and Nevill, 2003). DDT was effective in the eradication of blackfly larvae; however, this chemical was not species specific and resulted in mortalities of most of the macro-invertebrates in the river and this, compounded with the environmental consequences of its application, resulted in DDT being discontinued as a control option (Myburgh and Nevill, 2003). A shift away from DDT applications have given rise to the blackfly Control Programme within the Orange River.

2.4.2. Blackfly Control Programme larvicides

Blackfly abundance within the Orange River can be estimated using Palmer's (1994) 10-point visual scale method. This method was developed due to other methods being time consuming and to assist blackfly Control Programmes within the Orange River. Blackfly larvae and pupae found on rocks and trailing vegetation within the water are classed on a logarithmic density scale of 1-10 based on the diagrammatic representation seen in Palmer (1994), with 1 being the lowest abundant class and 10 being the highest abundant class. This method was shown to be accurate as estimates based on this method showed no significant difference from the physical counts of blackfly larvae and pupae taken (Palmer, 1994). Blackfly outbreaks are generally expected when the larvae found in stones-in-current habitat is a class 7 or greater mean abundance (Palmer, 1994; Rivers-Moore *et al*., 2014). This scale is used when weekly blackfly monitoring is undertaken by the Department of Agriculture, Forestry and Fisheries (DAFF) to provide a rapid assessment of blackfly abundance. Should there be high scores that indicate a high possibility of outbreaks, then control measures are recommended. Larvicide applications is the control method used by management to control blackfly numbers and preventing outbreaks.

Traditionally, the two types of larvicides applied along the Orange River have been *Bacillus thuringiensis var. israelensis* (Bti) which is a bacterial larvicide, and temephos which is an organophosphate (Palmer and Palmer, 1995; Myburgh and Nevill, 2003; Grey *et al*., 2012). The registered Bti and temephos for the blackfly Control Programme goes by the tradename Teknar® and Vectobac® for the Bti's and Abate® for the temephos (Palmer and Palmer, 1995; Rivers-Moore *et al*., 2014). Larvicide applications are generally applied by helicopters (Palmer and Palmer, 1995; Rivers-Moore *et al*., 2008a), which pilots are provided with information regarding the breeding sites and the volume of larvicide to be applied at each site (Rivers-Moore *et al*., 2008a).

Larvicides that are ecologically safe towards other non-target species within the river system are needed. According to Palmer and Palmer (1995), Bti's are considered favourable and was determined safe to most non-target species within river systems. Temephos was considered reasonably safe towards non-target species when the recommended dosages were followed, however, should temephos be applied at high dosages then there could be major impacts on non-target species (Palmer and Palmer, 1995). Temephos overdosing can lead to predator species being killed, which can lead to blackfly problems that is greater than what they were before larvicide application (Palmer and Palmer, 1995). Palmer and Palmer (1995), highlight the effects of both Bti's and temephos on non-target species through studies undertaken in Africa to eradicate the 'River Blindness' disease transmitted by blackflies. The consensus was that Bti's caused slight changes in the aquatic fauna, whilst temephos initially killed more than 50% of non-target species after the first application, however this figure declined after subsequent use, and temephos was determined to have little medium-to-long-term impacts on aquatic fauna.

The current larvicide used along the Orange River is Vectobac®, and to target the blackfly larvae with higher fecundity in winter, doses are doubled in concentration compared to doses applied in summer (10 000 ℓ and 5000 ℓ respectively) (Rivers-Moore *et al*., 2014). Bti larvicides are generally in the form of insecticidal crystal proteins (ICPs) and must be ingested to result in mortality amongst blackfly larvae (Gray *et al*., 2012). Bti's are effective towards filter feeding macro-invertebrates such as blackfly and mosquitoes (Myburgh and Nevill, 2003). Spores are produced by Bti's and contain the toxins which affect the larvae. Ingestion of these types of larvicide causes ruptures in the stomach walls of the host which results in paralysis and death (Myburgh and Nevill, 2003). Although Bti's are considered safe, the effectiveness of this larvicide might be limited within the Orange River. Bti's are believed to be most effective in clear water as the effectiveness of this larvicide is dependent on blackfly larvae being able to filter out a significant number of spores (Myburgh and Nevill, 2003). In clear water, spores are not diluted by silt particles or algae. The majority of the Orange River is turbid, characterised by algae covered rocks and suspended silt particles. This will impact on the larvae's ability to filter spores which will decrease effectiveness and mortality. It is recommended that Bti applications be restricted to river systems that have low flow rates (< 100 $m^3.s^{-1}$) (Myburgh and Nevill, 2003). The Orange River has many riffles and rapids where flows generally exceed 100 $m^3.s^{-1}$, which is generally ideal for blackfly populations (Rivers-Moore *et al*., 2014). Bti's can be applied to areas of high flow, however, this would require higher dosages and associated costs (Myburgh and Nevill, 2003).

This brings into question why Bti is the main larvicide used in the blackfly Control Programme when temephos has been successfully used in other blackfly control programmes as seen within *Onchocerciasis* control programmes within West African Rivers (Palmer and Palmer, 1995; Myburgh and Nevill, 2003). One of the advantages of these types of larvicide is that it can be used effectively in rivers that are turbid (Myburgh and Nevill, 2003). These types of larvicides can be absorbed onto silt particles which are filtered out by the blackfly larvae and stored in its gut which results in mortality (Myburgh and Nevill, 2003). Temephos can be applied to rivers where flow rates are high (> 300 $m^3.s^{-1}$) (Myburgh and Nevill, 2003). This supports the reasoning as to why temephos products such as Abate® should be the primary larvicides applied along the Orange River. However, due to misuse of these larvicides, Abate® has been deregistered for use along the Orange River which is why Bti larvicides are being used for control (Rivers-Moore pers comm., 2015).

2.4.3. Integrated Control

Integration of control measures with larvicide applications should be considered as an option for control for potential effectiveness and cost efficacy. Whilst larvicide applications are the preferred control measure used in the blackfly control programme of the Orange River, there are costs to this approach (Palmer and Rivers-Moore., 2008). Even though there are benefits to larvicide applications, dosages throughout the year are an expensive option if not done optimally (Rivers-Moore *et al*., 2008a). With annual larvicide applications, there is an issue that species will become resistant to such larvicides which will result in unsuccessful control and likely outbreaks (Palmer and Rivers-Moore., 2008). Blackfly larvae are significantly influenced by changes in flow and therefore flow manipulation should be a method explored further in achieving blackfly control (Myburgh and Nevill, 2003; Rivers-Moore and de Moor, 2008). Combining larvicide applications with flow manipulation might be a successful method of reducing larvicide costs and preventing species from becoming resistant to larvicides.

Flow manipulation is favourable within rivers that are heavily regulated, as they provide flow variability that do not naturally occur anymore due to the construction of dams, impoundments and IBTs (Rivers-Moore and de Moor, 2008). For flow manipulation, impoundments are needed above (upstream) of the identified blackfly breeding sites (Rivers-Moore and de Moor, 2008). Optimal time that flow manipulation should occur is winter, which is when the highest blackfly population of larvae occurs (Rivers-Moore and de Moor, 2008). Reductions during winter result in low flow or even no flow which will result in the substrate being exposed, which will dry out the blackfly larvae and pupae that are attached to it (Myburgh and Nevill, 2003; Rivers-Moore and de Moor, 2008). Winter manipulation is aimed to control the population that is expected in the following spring period and beneficial to farmers and stakeholders as it does not disrupt irrigation demands (Rivers-Moore and de Moor, 2008).

However, success via flow manipulation cannot be achieved in all river systems. Systems such as the Orange River has a complex use of water amongst various stakeholders, and there are large distances between impoundments which makes flow manipulation impractical (Rivers-Moore and de Moor, 2008). Challenges within the Orange River to implement flow manipulation as a control option is the reason why larvicide applications are currently the sole control method for the blackfly control programme (Rivers-Moore and de Moor, 2008). Integration with other control measures such as biological control is limited. Predators such as hydropsychids that feed on blackfly larvae are not effective enough to control high numbers of blackfly larvae (de Moor, 1989). When blackfly larvae (*S.chutteri*) emerge and are most abundant in the early spring season, the hydropsychids are absent from the system as they only emerge in the later stages of spring (de Moor, 1989). By the time they emerge the blackfly larvae abundance far outweighs that of the hydropsychid abundance, which renders their effectiveness of blackfly control limited (de Moor, 1989). Therefore, integrated measures are sound in theory but not viable in all situations.

With the limitations faced with integrated control measures along the Orange River, other options to reduce costs and larvicide resilience are required. Optimisation of larvicide dosages could be a potential method of reducing costs for the blackfly Control Programme (Rivers-Moore *et al*., 2008a). More efficient and effective applications of larvicides could occur when the relationship between volume of larvicides and downstream carry is understood (Rivers-Moore *et al*., 2008a). The estimated reduction in costs for the blackfly Control Programme range between R540 000 and R1 800 000 per annum when larvicides are used optimally depending on dosage and downstream carry (Rivers-Moore *et al*., 2008a). The understanding of downstream carry can be seen in applications from the Yellow River, Wisconsin, where *Simulium annulus* and *Simulium johannseni* were pest species within this system (Gray *et al*., 2012). The larvicide used was Vectobac® and applications 5.5km downstream showed a larval mortality of greater than 90% (Gray *et al*., 2012). Optimisation of larvicide application is achieved when less sites need to have larvicides applied to them as the larvicides applied at one site may carry downstream to the next. Being able to predict sites where outbreaks are most likely to occur would further reduce costs of larvicide applications, as specific sites could be targeted and dosed instead of dosing the entire river section. Predicting when in the year sites are most likely to experience outbreaks would potentially further reduce costs as larvicides could be applied in certain months instead of being applied on a regular basis, which in turn would prevent blackflies from becoming resilient to the applied larvicides.

2.5. Environmental Modelling

Even though the blackfly Control Programme is needed as it makes considerable economic sense (Palmer *et al*., 2007), it has not achieved its desired purpose as outbreaks periodically occur every five to ten years which can be attributed to poor implementation of control measures. Possible factors for why there are periodic outbreaks include; flow volumes and velocities that are higher than usual (Palmer *et al*., 2007), turbidity levels change which may result in switching of blackfly species (Fredeen, 1977; Rivers-Moore *et al*., 2014), larvicide resistance due to generational adaptions to repeated larvicides (Palmer and Rivers-Moore, 2008), and management challenges (Rivers-Moore *et al*., 2014). With climate change and rising global temperatures, it is expected that blackfly populations and outbreaks will increase, and therefore long-term solutions are needed as opposed to the short-term solutions as seen in previous studies (Palmer, 1994; Bonkewitzz and Palmer, 1997; Rivers-Moore *et al*., 2008a). While these studies are promising in reducing incidents of outbreak, is it vital that existing research be built upon in a long-term holistic manner which aims to reduce the probabilities of repeated outbreaks.

Recent research funded by the Water Research Commission (WRC) (Rivers-Moore *et al*., 2014) has shown promise in achieving this with the aid of a Bayesian network model (Bn). Bns provide a framework for management to assess uncertainty based on many competing and interacting variables (Kjaerulff and Madsen, 2008; Stewart-Koster *et al*., 2010). The Bn by Rivers-Moore *et al* (2014), was constructed to determine whether recent blackfly outbreaks that caused economic losses were caused by a switching in dominance from *S.chutteri* to *S.impukane*. This approach was successful in demonstrating that the cause of recent outbreaks was more likely to be a management-related issue than the result of species-switching from *S. chutteri* to *S. impukane*. The Bn showed the need to collect improved seston concentration and add a water temperature node. It additionally highlighted the potential for the following: being able to evaluate ongoing monitoring data through correlations with previous outbreak periods using the Bn, potential for climate change scenario analyses to assist management planning in the future and a public participation tool for transparency towards all stakeholders to promote joint problem-solving (Rivers-Moore and Palmer, 2017).

2.6. Bayesian network models (Bns)

Bayesian networks (Bn), also known as Bayesian belief networks (Bbns), are a method of modelling that has become increasingly applied over the past decade to integrate science and management, and to quantify ecological risks within aquatic environments (Uusitalo, 2007; Castelletti and Soncini-Sessa, 2007; Henriksen and Barlebo, 2008; McDonald *et al*., 2015). Bns is a scientifically credible approach when modelling complex systems in which uncertainties exist (Pollino *et al*., 2007; Henriksen and Barlebo, 2008). Popularity of Bns are due to the algorithms within the network that efficiently compute probabilities whilst not being limited to any structural restrictions (Aguilera *et al*., 2011). The ability to integrate multiple facets such as trade-offs, interactions and outcomes make Bns suitable for modelling environmental systems (Chen and Pollino, 2012). Stewart-Koster *et al*., (2010) identified the use of Bns as a rapidly growing model that can be used as a decision support tool or to model a specific system of interest. Modelling, with the use of Bns, of key ecological drivers and relationships can be beneficial to management in terms of potential restoration strategies for negatively impacted systems (Stewart-Koster *et al*., 2010). They are useful for understanding the influences of multiple variables on a response variable (Stewart-Koster *et al*., 2010; Rivers-Moore *et al*., 2014), as seen in previous studies on the Orange River on how flow discharge, seston concentration and channel type influences which blackfly species most likely to be found along the Orange River if those variables are observed to be in a certain state (Rivers-Moore *et al*., 2014).

Bns have proven to be useful for the facilitation of conceptual models when relationships between variables are attempting to be represented, even though there might be uncertainties between the relationships (Rivers-Moore *et al*., 2014). There is no reliance on large sets of empirical data such as other traditional statistical models, and can be used to classify or predict events with limited data (Uusitalo, 2007; Chen and Pollino, 2012). They are useful as they can handle missing data and allow the combination of various types of data including quantitative and qualitative data and include expert opinion (Uusitalo, 2007; Henriksen and Barlebo, 2008; Chen and Pollino, 2012). Chen and Pollino (2012), highlighted the case study where Bns and GIS were used to map habitat suitability for an endangered species which was based on limited empirical data and expert knowledge.

The blackfly Control Programmes has been in effect for the past few decades, however, environmental variables such as flow velocity, turbidity and water temperature have limited data, which is why Bns are ideal models to use in such systems and programmes. Another advantage of Bns is that the network can easily be updated when there is new knowledge or data available (Ticehurst *et al*., 2007), which can be active after blackfly monitoring is undertaken weekly. Bns can be used on a temporal scale as seen by the temporal models in the study by Pollino *et al*. (2007), and Bn structures must be flexible if wanting to apply for both spatial and temporal use. This is ideal for the Orange River system as blackfly outbreak probabilities can be predicted on a temporal scale, whilst outbreak probabilities at specific sites and sections of the river can be predicted on a spatial scale.

Bns can be modified and used potentially for management options (Stewart-Koster *et al*., 2010). These are called Bayesian decision networks (Bdns),and have additional decision and utility nodes within the network which show potential costs and benefits for actions taken (Stewart-Koster *et al*., 2010). Such networks are often used for environmental management, where the relationship between variables, such as environmental drivers and ecological response variables require modelling for viable solutions (Stewart-Koster *et al*., 2010). Networks which have states that are possible restoration actions, can have decision nodes included in them (Stewart-Koster *et al*., 2010). Decision nodes alter the states of the 'child' node that it is connected to, depending on the restoration action. Natural resource management programmes in south east Queensland Australia have utilised Bdns freshwater management by identifying various interacting variables within the system including climate change (Mantyka-Pringle *et al.,* 2016). The Bdn was created when decision, cost and utility nodes such as 'available management actions', 'cost of available actions' and 'benefit of management outcomes' were modified to the Bn (Mantyka-Pringle *et al.,* 2016). Bdns could be used for management options for the blackfly Control Programme as costs and benefits could be determined for potential actions taken.

Aguilera *et al*., (2011) highlights limitations associated with Bns which include: parameter estimations require more data as variable numbers increase; the available data for most studies are continuous and not discrete, and Bn software for commercial use have not incorporated fuzzy models even though it has been noted that Bns can handle these models. Bns deal with continuous data in a limited manner and this poses a challenge since continuous data are common in most studies (Uusitalo, 2007; Aguilera *et al*., 2011). However, this can be overcome with the discretisation of the variable which can be achieved within the Bns (Uusitalo, 2007). Expert knowledge within Bns is possible, however, there are two main concerns with incorporating expert knowledge into Bns highlighted by Uusitalo (2007). The first concern is that experts are not used to providing numbers and probabilities without relying on real data in which they draw values. The second concern is that experts are familiar with classical statistical analysis such as confidence intervals and point estimates rather than distributions which is why there might be scepticism towards Bns. Another limitation noted by Chen and Pollino (2012) was that Bn do not have the ability to represent feedback loops and relationships that are dynamic which would be useful for certain studies. Spatial and temporal dynamics can be modelled in Bns, however, it is time consuming and tedious as separate networks are needed for each time slice (Uusitalo, 2007).

2.6.1. Conditional structures of Bayesian networks (Bns)

Bayesian networks (Bns) are modelling techniques that are formed upon the basis of Bayes theorem, and provides reasoning for scenarios and conditions where uncertainty exists in a mathematically efficient way (Uusitalo, 2007; Castelletti and Soncini-Sessa, 2007; Henriksen and Barlebo, 2008; McDonald *et al*., 2015). Aguilera *et al* (2011) describes Bns as a statistically multivariate model for variables within the network. Bns have two components associated with it, namely the Qualitative and Quantitative components, where the qualitative component of the model is a Directed Acyclic Graph (DAG) which indicate linkages and dependencies between variables (Figure 2.1), whilst the quantitative component deals with conditional distributions of each variable within the network (Figure 2.2, 2.3 and 2.4) (Aguilera *et al*., 2011).

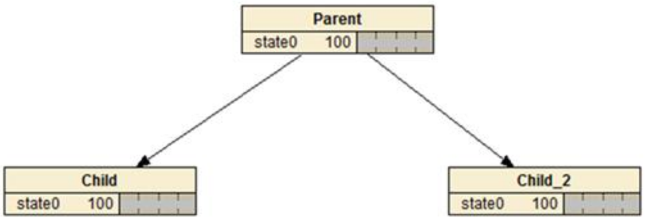

Figure 2.1: Casual Relationship between 'Parent' and 'Child' nodes.

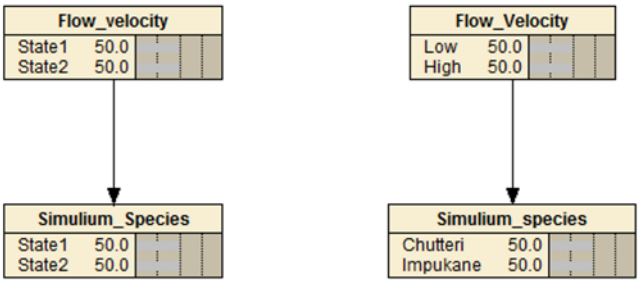

Figure 2.2: States renamed for 'parent' and 'child' nodes.

Turbidity	Flow_velocity	Channel_Type	Water_Temperature	Chutteri	Impukane
Low	Low	Anastomosing	Low		
Low	Low	Anastomosing	High		
Low	Low	Single	Low		
Low	Low	Single	High		
Low	High	Anastomosing	Low		
Low	High	Anastomosing	High		
Low	High	Single	Low		
Low	High	Single	High		
High	Low	Anastomosing	Low		
High	Low	Anastomosing	High		
High	Low	Single	Low		
High	Low	Single	High		
High	High	Anastomosing	Low		
High	High	Anastomosing	High		
High	High	Single	Low		
High	High	Single	High		

Figure 2.3: Unpopulated Conditional Probability Table (CPT).

Turbidity	Flow_velocity	Channel_Type	Water_Temperature	Chutteri	Impukane
Low	Low	Anastomosing	Low	50.677	49.323
Low	Low	Anastomosing	High	93.032	6.968
Low	Low	Single	Low	59.601	40.399
Low	Low	Single	High	55.127	44.873
Low	High	Anastomosing	Low	35.207	64.793
Low	High	Anastomosing	High	29.357	70.643
Low	High	Single	Low	98.337	1.663
Low	High	Single	High	24.201	75.799
High	Low	Anastomosing	Low	24.326	75.674
High	Low	Anastomosing	High	41.524	58.476
High	Low	Single	Low	59.599	40.401
High	Low	Single	High	44.821	55.179
High	High	Anastomosing	Low	70.727	29.273
High	High	Anastomosing	High	46.46	53.54
High	High	Single	Low	33.91	66.09
High	High	Single	High	42.993	57.007

Figure 2.4: Populated Conditional Probability Table (CPT).

2.6.2. Directed Acyclic Graph (DAG)

Bns are graphically illustrated as Directed Acyclic Graphs (DAG), consisting of nodes which represent variables of interest, and directed links called arcs between the nodes which identify direct dependencies amongst the variables (Figure 2.1) (Sprites *et al*., 2000; Uusitalo, 2007; Ticehurst *et al*., 2007; Chen and Pollino, 2012). The structure of the DAG is one of the most important advantages of Bns, as it determines whether the relationship amongst variables are dependent or independent, which is useful when attempting to identify relevance for a variable of interest without numerical calculations (Aguilera *et al*., 2011). Nodes that are not connected by arcs indicate conditional probabilistic independence from one another (Castelletti and Soncini-Sessa., 2007). Nodes within the Bn are identified as either a 'parent' or 'child' node (Figure 2.1) (Castelletti and Soncini-Sessa., 2007; Stewart-Koster *et al*., 2010). Castelletti and Soncini-Sessa (2007) explain the dynamics of the 'parent' and 'child' node using two example nodes named node A and node B, where there is an arc from A to B, which indicates that A has a causal influence of B, therefore A is the 'parent' node whilst B is the 'child' node. The arc connections between 'parent' and 'child' nodes are depictions of dependencies, which result in the DAG (Ticehurst *et al*., 2007;

Stewart-Koster *et al*., 2010). DAGs are representative of causal relationships amongst random variables within the Bns (Spirtes *et al*., 2000; Uusitalo, 2007; Rivers-Moore *et al*., 2014). It must be noted that there are no feedback loops within the DAGs, as the paths do not start and end at the same nodes (Stewart-Koster *et al*., 2010).

2.6.3. Nodes and States

Nodes have a number of possible outcomes associated with them, called states (Figure 2.2) (McDonald *et al*., 2015). States can be discrete and their values are mutually exclusive which are representative of the nodes possible conditions (Castelletti and Soncini-Sessa., 2007). Nodes can have a varying number of states, and the higher number of states associated with a node, the more complex the network is, and vice versa (Chen and Pollino, 2012). When the probabilities of the states of the 'parent' nodes are specified, the Bn undergoes a process of belief updating, meaning that the probabilities of the 'child' nodes are updated (Stewart-Koster *et al*., 2010; Chen and Pollino, 2012). This means that inference can be made, on the basis that Bns have causal relationships between 'parent' and 'child' nodes, that probabilities of the different states within the independent 'parent' nodes will have an influence on the probabilities of different states of the dependent 'child' nodes (McDonald *et al*., 2015).

'Parent' nodes that have no in-coming arcs are conditionally independent (McDonald *et al*., 2015). Whereas 'child' nodes have in-coming and out-going arcs, and a 'child' node that only has out-going arcs are called output 'child' nodes (McDonald *et al*., 2015). Again, drawing upon the theoretical Bn that was shown by Rivers-Moore *et al*., (2014), the network was designed to identify blackfly outbreaks along the Orange River, showed that the nodes 'Seston concentration', 'Discharge' and 'Channel type' were 'parent' nodes and conditionally independent, whilst the '*Simulium'* species node had in-coming and out-going arcs and therefore was a 'child' node (Figure 2.4). The 'Outbreak probability' node only had in-coming arcs and therefore is the output 'child' node. Estimation of any 'child' node probabilities is derived from the probability theory and Bayes theorem when the one of the state of 'parent' node is observed (Stewart-Koster *et al*., 2010). For the blackfly model, the 'parent' node 'Discharge' has two states which were identified as 'high' and 'low' (Rivers-Moore *et al*., 2014). Therefore, should the state of the 'Discharge' node be observed as either 'high' or 'low', this observation will influence the states of the '*Simulium'* species node, and the other 'parent' nodes will have the same effect.

2.6.4. Conditional Probability Tables (CPTs)

Nodes, states of nodes and directed arcs between nodes are conditional structures that are required within Bns. Once the conditional structure of the network is defined, it is important to know how strong the relationships among variables are, this is the basis of the quantitative component of Bns (Aguilera *et al*., 2011). After the conditional structures of the network have been determined, the model needs to be configured or learned from Conditional Probability Tables (CPTs) (McDonald *et al*., 2015). CPTs can specify degrees of belief, for which a node will be in a particular state depending on the states of the 'parent' node (Chen and Pollino, 2012).

CPTs are specified for each node, except for conditionally independent 'parent' nodes (root nodes) (Castelletti and Soncini-Sessa, 2007). CPTs require a *priori* data which populates the Bn for it to be quantified (Figure 2.3 and

Figure 2.4) (Stewart-Koster *et al*., 2010). A *priori* data can be quantitative or qualitative knowledge (or a combination of both) for variables within the network that are known prior to the development of the model (Uusitalo, 2007; McDonald *et al*., 2015). Expert knowledge can be used to specify the CPTs, or depending on the complexity of the network, CPTs can be specified by various learning algorithms (Stewart-Koster *et al*., 2010). A requirement of a Bn is the assumption of the Markov property, which means that populating each CPT should only be done by considering the direct 'parent' nodes of the 'child' node that is being quantified (Stewart-Koster *et al*., 2010).

CPTs do not have causal relationships for each variable, which is unlike the nodes within the network which are dependent and have a causal relationship 'parent' nodes (McDonald *et al*., 2015). This is beneficial as each node can be learnt and updated individually from a *priori* data when the network is being updated or developed further (Castelletti and Soncini-Sessa, 2007; McDonald *et al*., 2015). Examples of populating CPTs from a *priori* data were done McDonald *et al* (2015), in south-eastern Australia, where a *priori* data were collected from a short-term river health monitoring program, and CPTs were populated using Netica4.16 software. Data collected included; chlorophyll, Nitrogen oxides (NO_x) and Soluble Reactive Phosphate (SRP) which were used for the population of the CPTs (McDonald *et al.,* 2015). There are studies where field monitoring data were not used to populate the CPTs of the network as seen in the study by Ticehurst *et al*. (2007) and Bonafede and Giudici (2007). CPTs were populated through a combination of means including information from literature and expert opinions when quantitative data were not available (Ticehurst *et al*., 2007; Bonafede and Giudici 2007). Quantitative data collected from Orange River to populate the CPTs of the BN would help validate the model's accuracy.

2.7. Stakeholder Engagement Process

Stakeholder involvement is key to creating an interdisciplinary process for natural resource management (Chen and Pollino, 2012). When designing a Bayesian network (Bn) that will be used in other programmes or for management purposes, it is advised that stakeholders be involved in identifying the network structure (Ticehurst *et al*., 2007; Chen and Pollino, 2012). This is beneficial as knowledge and expertise from different sectors of society can be used and combined to ensure that a more complete understanding of the system be considered, and to ensure a common purpose between stakeholders and modellers (Henriksen and Barledo, 2008; Chen and Pollino, 2012). Bns can be designed so that stakeholders can use them to benefit their needs by updating data and frameworks, however, training and understanding of Bns are first required (Ticehurst *et al*., 2007). The Directed Acyclic Graph (DAG) is the component within the Bn which can be easily communicated to stakeholders and non-technical users (Henriksen and Barlebo, 2008). However, non-technical users and stakeholders might find difficultly in understanding the population and encoding methods of CPTs within the network, which is the most important component of the Bn (Henriksen and Barlebo, 2008). Therefore, stakeholder involvement within the construction of Bns may be limited to components to which they understand, which is why training may be required.

Good modelling practices are when all stakeholders within the modelled system are engaged with to formulate the most realistic and reliable model (Chen and Pollino, 2012). For good modelling practices to be achieved, careful considerations need to be taken during the construction, testing and application stages of the model (Chen and

Pollino, 2012). Henriksen and Barlebo (2008), highlight Bn constructions where there was integration of stakeholders and experts such as water managers to provide all possible information or knowledge on variables within the Bn. Seven steps were identified for the procedure of stakeholder involvement which were: 1) defining the study or context; 2) identification of factors, indicators or actions ; 3) construction of pilot networks; 4) data collection; 5) defining states within the network; 6) populating CPTs; and 7) gain feedback from stakeholders (Henriksen and Barlebo, 2008). This seven-step process was conducted over a series of workshops with regards to constructing a Bn for active groundwater protection. Other Bns where stakeholder involvement was prevalent in the construction of the model was identified by Pollino *et al*., (2007), where a Bn was constructed to assess how human activities have impacted the native fish communities within the Goulbum Catchment, Victoria, Australia. The aim of the study was to produce a model to support fishery management (Pollino *et al*., 2007). Workshops were held to engage stakeholders and experts to identify the communities of fish at risk and to establish the endpoints of the model and variables within the system (Pollino *et al*., 2007). This case study provides valuable insight and encouragement for stakeholder interactions when constructing Bns. However, Henriksen and Barlebo (2008) note that the stakeholder participation process could have limitations, as seen in their study where even though the process created a stakeholder dialogue which was beneficial, there was difficultly with attempting to persuade experts, stakeholders and citizen to accept Bn as a decision-making tool as uncertainties and scepticism was observed during the workshops (Henriksen and Barlebo, 2008).

For the research, stakeholder engagement with regards the blackfly outbreak Bn was required, as it was identified that there needs to be an alignment in views and priorities amongst all stakeholders affected by blackfly outbreaks. With farmers being the key stakeholders affected, it was vital to get them involved in the process. The Department of Agriculture, Forestry and Fisheries (DAFF) who conduct the blackfly Monitoring Programmes were identified as being another key stakeholder that should be involved in the Bn construction and implementation process.

2.8. Conclusion

Blackflies are cosmopolitan organisms that belong to the family *Simuliidae* (Kazanci, 2006). Their development stages (larval and pupal) limits them to water systems, as adults lay the eggs in water where they develop until they emerge as adults. Blackfly larvae and pupae distribution is generally species specific as some species prefer swift flowing turbid water, whilst others prefer slower flowing clear waters. Adult females require a blood meal to complete ovarian development (de Moor, 1989; Myburgh and Nevill, 2003), and livestock, poultry and humans are bitten to achieve this. The economic and health concerns that outbreaks pose are primary reasons behind blackfly Control Programmes being initiated in South Africa. The Orange River system has suffered severe economic losses within the sheep and wine industries during periods of blackfly outbreaks, with the pest species in this system being predominantly *Simulium chutteri (S.chutteri)* (Rivers-Moore *et al.,* 2014). This species is dominant due to being able to breed throughout the year and favouring high velocity flows and seston concentrations which are prevalent along the Orange River. Changes in flow regimes and reduced seasonality caused by the construction of dams, impoundments and Inter-Basin Transfer (IBTs) schemes are the main reason as to why blackfly outbreaks have occurred within South Africa over the past 40 years (Myburgh and Nevill, 2003; Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2013a).

While blackfly Control Programmes regularly apply larvicides to prevent outbreaks, outbreaks still periodically occur. Developing long term solutions is the key due to the anticipated threat such as increasing temperatures and climate change has on increasing populations and frequencies of blackfly outbreaks. Previous research has suggested that the use of Bayesian network models (Bns) could potentially be a solution. Bns are well suited to modelling systems such as the Orange River, with limited long-term data and various stakeholders. Construction of conditional structures are important, and stakeholders must to be involved in this process to provide expertise and opinions which would help validate the accuracy of the model. Identification of key variables for the Bn is needed and collection of data for the CPTs of the model are required. To develop a working model of blackfly outbreak probabilities at selected sites along the middle to low sections of the Orange River, which could potential benefit control managers and affected farmers. Modifying Bns into Bayesian decision networks (Bdns) could be a pro-active management tool when identifying costs and benefits of actions taken. Bns construction that would refine prediction accuracy along segments of the Orange River is the purpose of this research.

Chapter Three

Methods

3.1. Introduction

This chapter describes the abiotic and biotic data collected from 14 sample sites along the middle to lower reaches of the Orange River to aid in the construction of the Bayesian networks (Bns). Data were collected during four sampling periods from November 2015 to December 2016. This research is based on the preliminary Bn developed by Rivers-Moore *et al*. (2014) which identified key variables towards the probability of blackfly species distribution and outbreaks. Adaptions were made from this Bn and additional nodes included which was needed to refine blackfly outbreak probabilities generated from the Bns. With the blackfly Control Programme focused on the middle to lower sections of the Orange River which is approximately 850km in length, there are an estimated 148 sites that have been identified as suitable blackfly breeding sites (Rivers-Moore *et al*., 2008a; Rivers-Moore *et al*., 2014). Site selection was based on previous sample sites used by Rivers-Moore *et al.,* (2014) or monitoring sites used by the Department of Agriculture, Forestry and Fisheries (DAFF) for which they conduct weekly blackfly larvae and pupae monitoring.

3.2. Study Sites

The Orange River is the largest river system in South Africa and is approximately 2300km in length (Heyns, 2003). The source is located within the Lesotho Highlands and extends to Alexander Bay, Atlantic Ocean. There are three major storage reservoirs along the Orange River namely the Gariep, Vanderkloof (both located within South Africa) and the Katse dam (located in Lesotho). The Gariep and Vanderkloof dams are the largest and second largest reservoir storages in South Africa. These supplies are used for irrigation and for hydro-electric production. There are several water transfer schemes taking place along the Orange River to provide various sectors with assured water. A total of fourteen sites were sampled along the middle to lower reaches of the Orange River where biotic and abiotic data were collected (Figure 3.1). Many of the sites are located within the towns of Upington and Keimos with other sites located further away within the towns of Prieska, Douglas and Blouputs (Figure 3.1). Elevations ranged from 993m (Site 1) to 439m (Site 11).

At each site, various hydraulic habitats were sampled for blackfly larvae and pupae as it was anticipated that different blackfly species would be found in different habitats (Palmer, 1997; Palmer and Craig, 2000; Rivers-Moore *et al*., 2014). This was important as blackfly species collected and identified to be the most likely to cause an outbreak would be used within the Bayesian networks (Bns) as states for the node *Simulium* (Table 3.2). In addition, varying habitats were useful to obtain a comprehensive dataset of environmental conditions and species to determine species thresholds for 'parent' node state population and for Conditional Probabilities Tables (CPTs) within the Bns. Sites clustered in Keimoes were expected to have varying habitats due to the anastomosing nature of this segment of the Orange River. In this segment of river, the underlying geology results in splitting from a single channel system to a multiple channel system in which flow velocities and volumes are reduced within each individual braid of river (Rivers-Moore *et al*., 2013b).

The four periods where sampling was conducted were in November 2015, March 2016, July 2016 and December 2016. Sampling periods were conducted when transport and accommodation were available to the Northern Cape, with each sampling period aiming to be seasonally different from the other to determine variations in abiotic and biotic data.

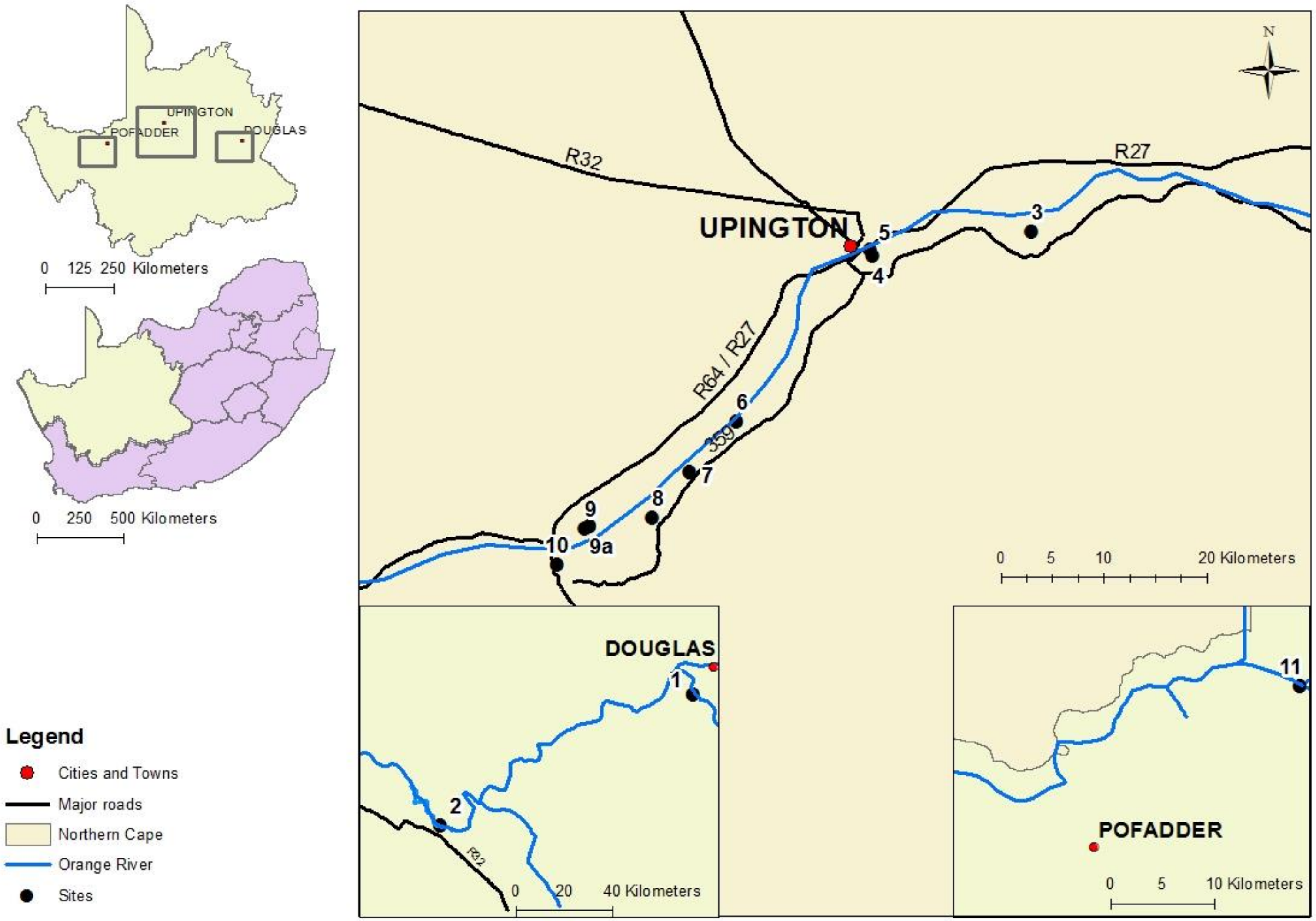

Figure 3.1: Site map indicating samples sites along the middle to lower sections of the Orange River, Northern Cape Province, South Africa.

Table 3.1: Physical characteristics of sample sites along the middle to lower reaches of the Orange River.

Site	Site name	Elevation (msl)	Notes and GPS coordinates:
1	Douglas	993	Main Channel- Under Douglas bridge. (-29.16194 S 23.69623 E)
2	Prieska	926	Main Channel- Under Prieska bridge. (-29.65553 S 22.74592 E)
3	Giflkloof	800	Main Channel- DAFF monitoring site. (-28.43743 S 21.40092 E)
4	UP1	785	Main Channel- Under Upington railway bridge. (-28.45798 S 21.26165 E)
5	UP13	785	Main Channel- By Sun River Lodge. (-28.45262 S 21.25943 E)
6	Druiswater	778	Main Channel- Private farm. (-28.60385 S 21.14277 E)
7	UP6	765	Main Channel- Kanoneiland. (-28.46768 S 21.10197 E)
8	UP8	746	Main Channel. (-28.68780 S 21.06878 E)
8a	UP8-Stream	746	Return flow side stream. Natural, shaded stream. (-28.68780 S 21.06878 E)
8b	UP8-Channel	746	Agricultural return flow canal. (-28.68780 S 21.06878 E)
9	UP12	726	Braided channel. (-28.69490 S 21.01452 E)
9a	UP2_Soverby	726	Side stream adjacent to braided channel. (-28.69490 S 21.01438 E)
10	Ikaia	724	Main Channel- Ikaia lodge. (-28.72913 S 20.98595 E)
11	Blouputs	439	Main Channel. (-28.51377 S 20.18694 E)

3.3. Data collection

Abiotic and biotic data were collected during all four sampling periods from all sites. Data were collected with the purpose to aid the construction of the Bayesian networks (Bns). Abiotic data were collected to determine the spatial resolutions of the Bns. Biotic data (blackfly) were collected and identified which blackfly species are most likely to cause an outbreak and to add as states within the Bns for relevant nodes. Both abiotic and biotic data were used when creating the case files used for the Conditional Probability Tables (CPTs) of each spatial Bn.

3.3.1. Abiotic data

In-stream abiotic data were collected at each site to obtain a dataset of the environmental conditions and thresholds of the collected blackfly species. The abiotic data collected were; flow velocity ($m.s^{-1}$), water clarity (cm) (which were converted to seston concentration), water temperature (^oC), pH and conductivity. Flow velocity was collected to determine blackfly species thresholds and for case files inputted into the Conditional Probability Tables (CPTs) within the Bayesian networks (Bns), but was not used to determine spatial resolutions of the Bns which were done with seston concentration, water temperature, pH and conductivity data.

3.3.1.1. Flow velocity

Flow velocity measurements were recorded for sites at which blackfly larvae and pupae where sampled and scored with the use of the Transparent Velocity Head Rod (TVHR) (Fonstad *et al.,* 2005) (Plate 3.1). To obtain velocity readings from the TVHR, the rod is placed perpendicular into the river's surface and flow. The observed maximum and minimum height that the water reaches on the rod were recorded and the difference in these values (known as the change in height) were used to determine the flow velocity ($m.s^{-1}$) with the aid of a table of calculated velocities (Appendix 1). Flow velocities were used to establish thresholds based on flow velocity versus blackfly species abundance. Critical threshold values, determined for flow velocity, were converted to discharge ($m^3.s^{-1}$) values, with the aid of discharge versus flow velocity curves for the Orange River by Palmer (1997).

3.3.1.2. Seston concentration

Seston concentration affect blackfly species composition and abundance (Palmer and Craig, 2000; Rivers-Moore *et al*., 2014), and was converted after clarity readings were collected using a Water Clarity Tube (Dahlgren *et al.,* 2004) (Plate 3.1). A total of three water clarity readings (cm) were taken at each site per sampling period. The water clarity readings (cm) were converted to seston concentration readings ($mg.\ell^{-1}$) using equation 1 (Palmer, 1997; Rivers-Moore *et al*., 2007). As with the flow data, the seston concentration readings were collected at all sites to determine thresholds for recorded blackfly species.

[Equation 1]

Water clarity (cm) to seston concentration ($mg.\ell^{-1}$) equation:

Seston concentration = $10^{(\mathrm{Log10}(x / 256) / -0,616)}$

Where water clarity = x

3.3.1.3. Water temperature

Hourly water temperature data were collected at each site, as water temperature affect life cycles and exasperate outbreaks (Rivers-Moore *et al*., 2013a). To obtain a comprehensive dataset of daily average water temperature and fluctuations, HoboTidBit version 2 water temperature data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) were installed at all sites. Data were downloaded from these loggers when the sites were revisited. Water temperature data were collected hourly at 13 sites (site 8b was excluded) between 4 November 2015 to 5 December 2016. Hobo loggers at sites were not installed all at the same time and therefore not all sites had a full dataset starting from 4 November 2015 (Table 3.2). Therefore, data had to be patched by linear regression relationships from data against the first installed logger which was at site 5. For sites with unknown water temperature values due to installation delays, regressions were determined for collected data from each site with data from site 5 to obtain constant and beta data for each site. Data were patched for unknown data with the following equation: Unkown value = Site Constant + (Site Beta × Site 5 water temperature value).

Table 3.2: Installation dates and activated times of HOBO loggers to collect water temperatures at each site.

Sites	Date installed	Time activated
1	06 March 2016	14:00
2	08 November 2015	13:00
3	05 November 2015	12:00
4	06 November 2015	16:00
5	03 November 2015	0:00
6	06 November 2015	12:00
7	04 November 2015	16:00
8	07 November 2015	12:00
8a	07 November 2015	12:00
9	05 November 2015	15:00
9a	10 March 2016	10:00
10	04 November 2015	14:00
11	11 March 2016	12:00

3.3.1.4. pH and conductivity

The pH and conductivity ($\mu S.cm^{-1}$) readings were obtained with a Hanna pH/conductivity probe. A total of three readings were taken per site per sampling period. These variables were used to establish thresholds for blackfly species. Should pH and/or conductivity show clear thresholds that these variables affect blackfly species composition and abundance, it would be included as nodes within the Bayesian networks (Bns). However, if not then it would be excluded as a node within the Bns but still used to determine ordination split used for the spatial resolutions of the Bns.

Plate 3.1: Velocity readings using the Transparent Velocity Head Rod (TVHR) (Left); Water clarity readings using the Water Clarity Tube (Right).

3.3.2. Biological data

Blackfly larvae and pupae were collected based on the principles from de Moor's (2003) guide on collection, rearing and preservation (Plate 3.2). Different substratum was examined at each site for the presence of larvae and pupae. In-stream rocks and vegetation were the main substratum examined as these are considered the most suitable substrate for blackfly larvae and pupae attachment. Larvae and pupae were collected from these substrata at each site and had density scores assigned to them (Appendix 2). Blackfly larvae and pupae on these substrata were either collected and washed in 250 μm hand nets (rocks) so that the species would be collected in the bottom of the nets, or extracted directly with a fine pair of tweezers (trailing vegetation). Thereafter, larvae and pupae were placed in 250ml plastic bottles filled with 70% alcohol (for preservation) and were identified at a later stage with a dissecting microscope. A total of five samples were taken per site per sampling period to identify species composition and abundance which was used when constructing the Bayesian networks (Bns). Composition data were used to determine which species are most likely to cause an outbreak which were used as the states for relevant nodes, and scores and abundance data at each site were used when creating case files for the Conditional Probability Tables (CPTs) of each spatial model.

Plate 3.2: Searching in stream for blackfly larvae and pupae amongst vegetation and rocks (Left); Storing blackfly larvae and pupae in 70% alcohol for preservation (Right).

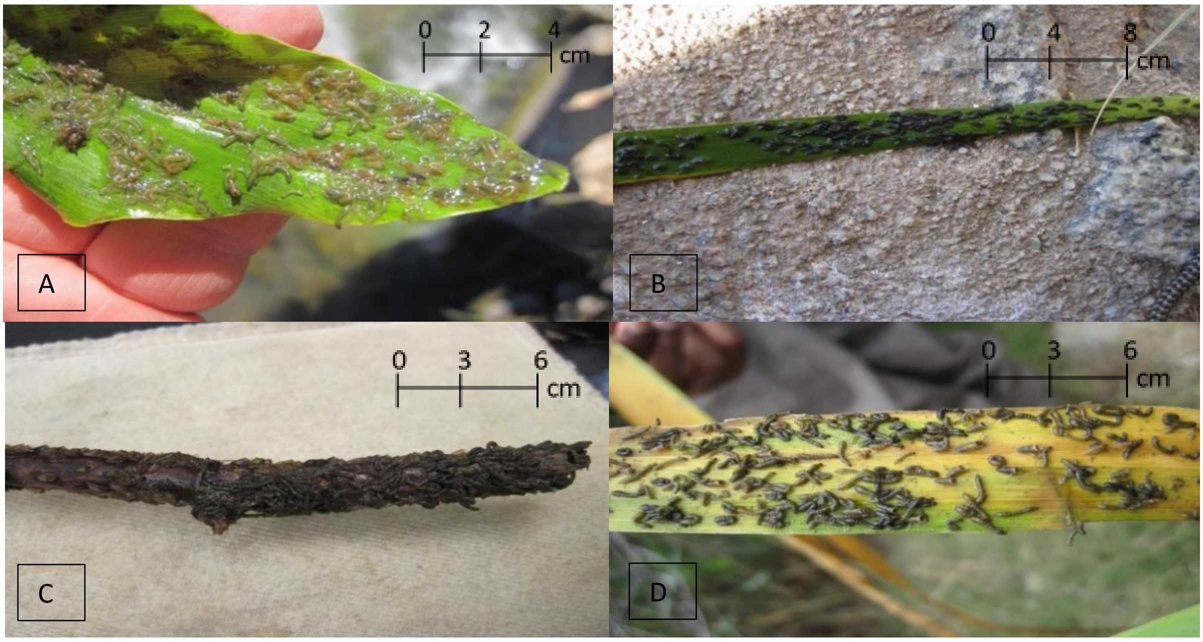

Plate 3.3: Blackfly larvae and pupae found on trailing vegetation which were scored on Palmer's (1994) abundance scale and placed in sample bottles for laboratory analysis. Density scores for all were scored above 7 which is indicative of a high density. A) Density score of 7. B) Density score of 7. C) Density score of 9 or 10. D) Density score of 7.

3.4. Bayesian network (Bn) development

3.4.1. Spatial and Temporal resolution of the Bayesian networks (Bns)

Bayesian networks (Bns) were used to predicted blackfly outbreak probabilities for the sample sites. To avoid a high number of unnecessary models (Bns), sites were grouped based on data collected during the four sampling periods. Principal Component Analysis (PCA) were used to group sites together and from this Bns were constructed for each grouping of sites. PCAs were run based on collected seston concentration, pH and conductivity to determine whether there is a split in ordination based on these variables. Furthermore, PCAs were run on water temperature data obtained from Hobo loggers at each site as this was the most complete and accurate dataset of all the variables collected and available. This was important as a large annual dataset may show more accurate splits in ordination than a smaller periodic dataset will.

The chosen temporal resolution for the Bayesian networks (Bns) was to run the models monthly, due to DAFF's blackfly monitoring being conducted on a weekly to monthly basis. Each model would generate 12 outbreak probabilities per year which was to identify which months are most likely to experience blackfly outbreaks for each model. However, should monthly probabilities show clear seasonal trends, outbreak probabilities could be refined to additionally show seasonal probabilities.

3.4.2. Nodes and States of the Bayesian networks (Bns)

The conditional structures of the Bayesian network (Bn) are the nodes of key variables identified to affect the response variable, which is blackfly outbreak probabilities. Table 3.3 refers to the nodes within the models. Once the nodes were identified, each node was assigned either as a 'parent' or 'child' node depending on the causal relationships they have with each other. Once the nodes for the Bn were determined, states were assigned to each node. Each node were discrete in nature and was assigned two states as multiple states would lead to a more complex network (Cain, 2001).

Table 3.2: Driver variables for the Bayesian networks (Bns).

Variable	**States**	**Justification**
Abiotic	Unfavourable/Favourable	Affects blackfly species type and density.
Algal blooms	Present/Absent	Algal films on rocks competitively exclude blackfly larvae and pupae.
Biotic	Strong/Weak	Affects available habitat for blackfly.
Channel type	Anastomosing/Single	Affects the type of blackfly species present (Rivers-Moore *et al*., 2013b; Rivers-Moore *et al*., 2014).
Discharge	Low/High	Affects the type and density of blackfly species present (Palmer and Craig, 2000; Rivers-Moore *et al*., 2013b; Rivers-Moore *et al*., 2014).
Larvicide efficacy	Optimal/Suboptimal	Larvicide success is dependent on whether conditions are optimal or suboptimal during application.
Management options	Effective/Poor	These can either be effective or poor based on whether outbreaks occur or not.
Outbreak Probability	Low/High	Is affected by all other variables.
Reeds	Absent/Present	Provide blackflies with available habitat.
Seston Concentration	Low/High	Affects the type and density of blackfly species present (Palmer and Craig, 2000; Rivers-Moore *et al*., 2013b; Rivers-Moore *et al*., 2014).
Simulium Group	Standard/Strong Porous	Groups based on labral fan structure (Palmer and Craig, 2000).
Spraying	Successful/Unsuccessful	Success or failure of spraying will influence whether management is successful or not.
Water temperature	Cool/Warm	Determines lifecycle length and number of generations per annum (Rivers-Moore *et al*., 2013a; Rivers-Moore *et al*., 2013b)

3.4.3. Population of state probabilities for 'parent' nodes

After the conditional structures of the Bayesian networks (Bns) were determined (nodes and states of nodes), probabilities and Conditional Probabilities Tables (CPTs) were populated. For each 'parent' node, probabilities were inputted for each of their states. State probabilities indicate what the likelihood of the node's state will be at any given time. Probabilities for state nodes were either calculated from data collected during sampling seasons or historical time series data, or if nodes had no available quantitative data, probabilities were populated when Conditional Probability Tables (CPTs) were populated. For nodes that had quantitative data available, return intervals were calculated for a threshold value dividing states to obtain probabilities. Return intervals estimates the likelihood of an event reoccurring such as the likelihood of a variable being above or below a certain threshold value.

Return intervals were calculated in Excel when data were inputted in chronological order and assigned numbers, after which the data and assigned numbers were sorted in ascending order. Spearman's rank and other ranking techniques were used on the sorted data to rank each of the data. Rank percentage (%) were then calculated with the following equation: Rank (%) = (Rank / Total number of data) × 100. Return intervals for each data value were then calculated with the following equation: Return interval = 1 / (1 – Rank (%) / 100). Value probabilities were then calculated by using their calculated return interval with the following equation: Probability = 1 / Return interval. Thereafter, critical values for nodes were used to obtain state probabilities.

There were six 'parent' nodes for which state probabilities were populated. These nodes were: Discharge, seston concentration, channel type, water temperature, reeds and spraying.

3.4.3.1. Discharge probabilities

With long term flow velocity ($m.s^{-1}$) data unavailable, discharge ($m^3.s^{-1}$) was used as a node within the Bayesian networks (Bns), as there is long term data accessible from the Department of Water Affairs (DWA) (https://www.dwa.gov.za/hydrology/HyStations.aspx?Region=D&StationType=rbRiver). Each sampling site did not have their own discharge data, therefore discharge data from nearest gauging weirs were deemed to be most applicable (Table 3.4). Once the spatial resolutions of the Bns were determined, the geographically closest gauging weir dataset was used for each Bn. Preference was given for gauging weirs that had the longest and most up-to-date data, therefore, gauging weir D7H005 would be given preference over gauging weirs D7H004 and D8H014 for example, as these weirs are either not update-to-date (D7H004) or do not have a long-term dataset (D8H014). Return intervals were calculated from the gauging weir data and state probabilities were populated within the Bns.

Table 3.4: Gauging Weirs for discharge ($m^3.s^{-1}$) data closest to sample sites.

Gauging Weir	Sites closest to weir	Flow data available from:
Upington D7H005	3,4 and 5	1936-10-01 to 2016-05-26
Kannon Island D7H004	6,7,8,9 and 10	1971-08-07 to 1989-02-20
Prieska D7H002	2	1959-05-01 to 2016-05-25
Marksdrift D3H008	1	1962-08-14 to 2016-05-25
Blouputs: D8H014	11	2014-01-29 to 2016-04-21

3.4.3.2. Seston concentration probabilities

Unlike discharge, historical seston concentration (mg.ℓ^{-1}) data were not readily available. The only seston concentration data available were the data collected from water clarity measurements taken during sampling periods and by the Department of Agriculture, Forestry and Fisheries (DAFF) whilst undertaking weekly blackfly monitoring. Data were converted to seston concentration (mg.ℓ^{-1}) from water clarity (cm) using equation 1 (page 29). Ideally, state probability calculation should be done from a long-term dataset rather than a short-term one. Therefore, Palmer's (1997) equation was used to convert discharge data ($m^3.s^{-1}$) from gauging weirs to seston concentration (mg.ℓ^{-1}) (Equation 2). Data from the same gauging weirs were used as for the discharge node. Validity of the converted discharge data were tested to see whether they represent accurate seston concentrations before used for node probabilities.

[**Equation 2]**

Discharge ($m^3.s^{-1}$) to seston concentration (mg.ℓ^{-1}) equation:

Seston concentration= 1.92 * $x^{0.755}$

Where discharge = x

3.4.3.3. Water temperature probabilities

Historical water temperature data were not available, however, with the installation of the HOBO loggers at each sampling sites, data collected were used to calculate state probabilities in the Bayesian networks (Bns). Although there was a dataset for all the sampling sites, only one set of data were required to calculate return intervals for each Bn. The sampling site data that were used would be the site that was closest to the gauging weir used in each Bn, from which discharge and seston concentration data were calculated.

3.4.3.4. Channel type probabilities

Calculation state probabilities for the node channel type were achieved with the aid of Google Earth. The total length (km) of anastomosing segments of river between sites within each spatial model was identified and used for the calculation of state probabilities. Anastomosing segments of river is where the single channel splits into multiple channels which significantly reduce flow volumes and velocity (Rivers-Moore *et al*., 2013b; Rivers-Moore *et al*., 2014). The start of each anastomosing segment to the point where it reverts into a single channel were measured and added together between sites, and was used to calculate probabilities with equation 3. A 1:50 000 map could be used for this process as opposed to Google Earth, however, Google Earth was chosen as it was deemed a quicker and more efficient method, and was used to precisely locate and orientate sample sites.

[Equation 3]

Anastomosing Channel Probability= Total anastomosing length / Total river segment length

Single Channel Probability = 1 – Anastomosing Channel Probability

3.4.3.5. Reeds and Spraying probabilities

There were no quantitative data available for the nodes ‘reeds’ or ‘spraying’. To populate state probabilities for these nodes state probabilities were populated automatically once the Conditional Probability Tables (CPTs) were populated with case files of data collected from the field.

3.4.4. Population of Conditional Probability Tables (CPTs) for ‘child’ nodes

For the ‘child’ nodes within the Bayesian networks (Bns), Conditional Probability Tables (CPTs) were populated for the model to function and to generate blackfly outbreak probabilities. The Bns were kept as simple as possible with a maximum of three ‘parent’ nodes linked to any given ‘child’ node. A high number of ‘parent’ nodes linked to a single ‘parent’ node would result in a very large CPT which would make a complex Bn. CPTs were populated when case files were inputted and populated through learning algorithms within the Bns. Case files were constructed for each spatial model based on data collected from sites during sampling periods from November 2015 to December 2016. Case files were constructed in Excel and consisted of all nodes within the Bns which were represented in columns and observed states for each of these nodes were populated in the rows (Appendix 3 and 4). Observed states of nodes were from the abiotic and biotic data collected during the four sampling periods. All entries in a single row were that of data collected at a specific point where sampling was undertaken. Therefore, each row was attributed to each sampling point where blackfly were scored and abiotic data collected for all four sampling periods to provide a large dataset which was used to generate the CPTs for each spatial model.

3.4.5. Model assumptions

When the case files were constructed for the Conditional Probability Tables (CPTs), certain assumptions were made as there was no available quantitative data for certain nodes. States within case files for the nodes algae, larvicide efficacy, management and spraying were populated based on assumptions derived from the state of their ‘parent’ nodes. Assumptions based off ‘parent’ nodes were thought to be appropriate due to the following reasons: algae is controlled by water and seston concentration (Palmer, 1997; Rivers-Moore and Palmer, 2017), larvicide efficacy is controlled by water temperature and seston concentration (Palmer, 1997; Rivers-Moore and Palmer, 2017) and management is a success based on larvicide efficacy and successful spraying (Rivers-Moore and Palmer, 2017). Therefore, should water temperature be observed to be warm and seston concentration be low, the assumption is that it is most likely that algae will be present and larvicide efficacy will be optimal. Whereas cool water temperatures and high seston concentrations will result in the assumption that there will be an absence of algae and sub-optimal larvicide efficacy. For management, an optimal larvicide efficacy and successful spraying results in the assumption that management will be effective, whereas sub-optimal larvicide efficacy and unsuccessful spraying results in the assumption that there will be poor management. For the spraying node, there was no ‘parent’ nodes to base assumptions on, blackfly abundances and scores were used for this assumption. Should there be low blackfly score and abundance at a point, the assumption would be that spraying was successful, however, should there be a high blackfly score and abundance at a point, the assumption would be that spraying was unsuccessful.

3.4.6. Model verification

Once the Bayesian networks (Bns) were constructed and outbreak probabilities generated, verification was carried out to ensure that these probabilities were accurate. To verify monthly outbreak probabilities, blackfly densities collected during sampling periods and blackfly monitoring data from the Department of Forestry and Fisheries (DAFF) was used. DAFF monitoring data from 2009 to 2016 consisted of blackfly density scores based on Palmer’s (1994) logarithmic scale (Appendix 1). Density scores were collated and average scores plotted for each month when monitoring was conducted for each year. For certain years, there was no monitoring for various months and therefore density scores were unknown for these months. Therefore, density scores of all years were plotted together to obtain a trend of monthly scores from 2009 to 2016, and this monthly trend compared and verify to the outbreak probabilities generated from the Bns.

3.4.7. Scenario analysis

Outbreak probabilities were generated based on current conditions along the lower to middle reaches of the Orange River. However, it is possible for these conditions to change particularly with climate change. Therefore, it is useful for management to understand how changes will impact outbreak probabilities. Outbreak probabilities from the Bayesian networks (Bns) were generated for the following scenarios: 1) an assumed 60% increase in discharge; 2) an assumed 2^{o} C increase in water temperature; 3) an assumed increase in discharge (60%) and water temperature (2^{o} C). Values are based on predicted changes in the future for discharge (Rivers-Moore and Palmer, 2017) and water temperature (Ragab and Prudhomme, 2002). These outbreak probabilities were compared to the outbreak probabilities based on current conditions.

3.5. Conclusion

Abiotic and biotic data were collected from 14 sampling sites along the lower to middle reaches of the Orange River. Sites ranged between the towns of Douglas to Blouputs. Abiotic data were collected to determine thresholds for blackfly species and to determine whether there is a split in ordination with the aid of Principal Components Analysis (PCAs) which were needed to identify whether sites are distinctly different or similar. Thresholds and critical values were used when calculating state probabilities with return intervals for the Bayesian networks (Bns) and PCAs were used to determine the spatial resolutions for the Bns. Biotic data collected were that of blackfly larvae and pupae and were counted and identified together to determine which *Simulium* complex groups are most problematic. Bns conditional structures were determined based on variables significant to blackfly abundance and distribution. Probabilities for ‘parent’ nodes states were populated through historical or available data. ‘Child’ nodes had Conditional Probabilities Tables (CPTs) populated through case files created based on data collected from the field. Monthly outbreak probabilities were generated for the Bns and verified with the aid of historical monthly DAFF monitoring scores to ensure the accuracy of the Bns. Outbreak probabilities were generated for various scenarios and were compared to the baseline probabilities under current conditions.

Chapter Four

Results

4.1. Introduction

This chapter presents abiotic and biotic data collected from four sampling visits, conducted in November 2015, March 2016, July 2016 and December 2016 at 14 sampling sites along the Orange River. The abiotic data presented were seston concentration (mg.ℓ^{-1}), pH, conductivity (μS.cm^{-1}) and water temperature (^{o}C) data. Biotic data was blackfly larvae and pupae collected at each site. Species thresholds for variables collected were determined and used for state probability calculations for nodes within the Bayesian networks (Bns). Spatial and temporal resolution of the Bns were determined from time series water temperature data collected from each site. Principal component analysis (PCA) and cluster analysis of water temperature data were used to determine whether sites were distinctly similar or different, and from these grouping spatial resolutions of the Bns were determined. Temporally, each model were run twelve times, for each month of the year, thereafter probabilities refined as they showed seasonal trends. Probabilities and Conditional Probabilities Tables (CPTs) were populated and are presented to provide outbreak probabilities generated by the Bns under current conditions and potential changes in conditions.

4.2. Abiotic data

4.2.1. Seston concentration

The highest seston concentration was at site 5 (Refer to Table 3.1 for site descriptions) which has maximum value of 62.7 mg.ℓ^{-1} (Figure 4.1). The lowest seston concentration were found at the side stream sites 8a, 8b, 9a with a low of 4.6 mg.ℓ^{-1} (Figure 4.1). The side stream sites had a much narrower range in comparison to the main stream sites (Figure 4.1). Overall, data collected at sites during November 2015 and March 2016 showed higher seston concentrations in comparison to July 2016 and December 2016.

4.2.2. pH

pH was consistent between sites and seasons with the pH of the system being slightly alkaline (Figure 4.2). The highest pH was found at site 8b (8.82) (Figure 4.2). Aside from the side stream sites, the main stream site with the highest pH was site 4 (8.81) (Figure 4.2). The lowest pH was found during July 2016 at site 9 (6.5) (Figure 4.2). The pH readings collected were constant between sampling periods, with only July 2016 being slightly lower.

4.2.3. Conductivity

The highest conductivities were found at site 8b (1531 μS.cm^{-1}) being the highest recorded (Figure 4.3). The lowest conductivity was found at site 2 (202 μS.cm^{-1}). Conductivities are similar, with only the side stream sites showing a higher overall conductivity than the main stream sites. Conductivities were observed to be highest during July 2016 and lowest during November 2015 and March 2016.

4.2.4. Water temperature

All sites showed similar seasonal trends with the lowest water temperature (^{o}C) experienced during the winter months of June and July and the highest water temperatures (^{o}C) experienced during the summer months of December and January. Overall, site 8a had the lowest mean water temperature of 17.5^{o} C (Figure 4.4). From the main river sites, site 1 had the lowest mean water temperature of 19.6^{o} C and site 11 had the overall highest of all sites (21.9^{o} C) during the sampling period (Figure 4.4). The minimum and maximum water temperature was at site 9a 5.5^{o} C and 42.2^{o} C respectively (Figure 4.4).

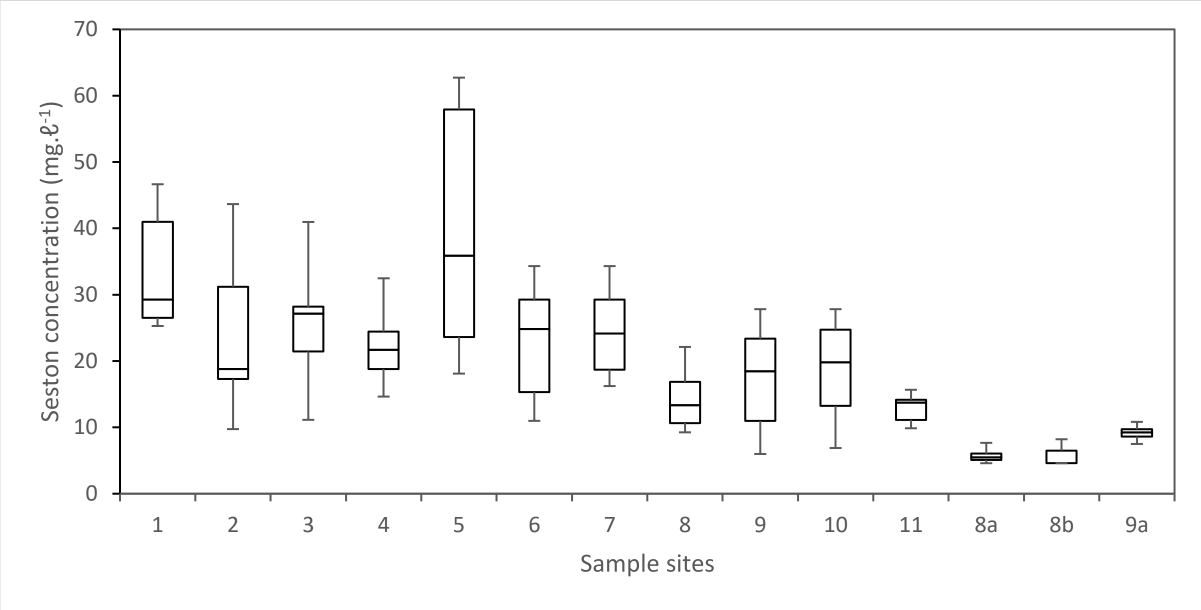

Figure 4.1: Box and whisker plot of Seston concentration (mg.ℓ^{-1}) collected during all field visits at sample sites going from upper stream to lower stream (sites 1 to 11), with the last three sites corresponding to side channels which are distinctly different from other sites (Table 3.1). The 'box' represents median and 25th/75th percentiles values and 'whiskers' representing maximum and minimum values.

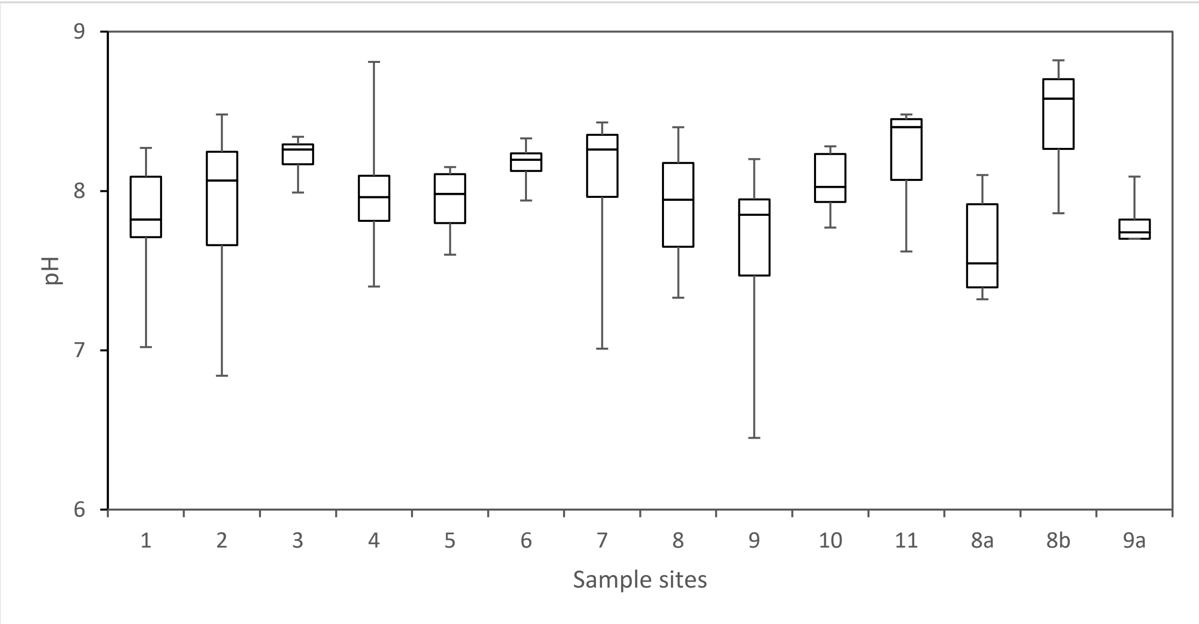

Figure 4.2: Box and whisker plot of pH collected during all field visits at sample sites going from upper stream to lower stream (sites 1 to 11), with the last three sites corresponding to side channels which are distinctly different from other sites (Table 3.1). The 'box' represents median and 25th/75th percentiles values and 'whiskers' representing maximum and minimum values.

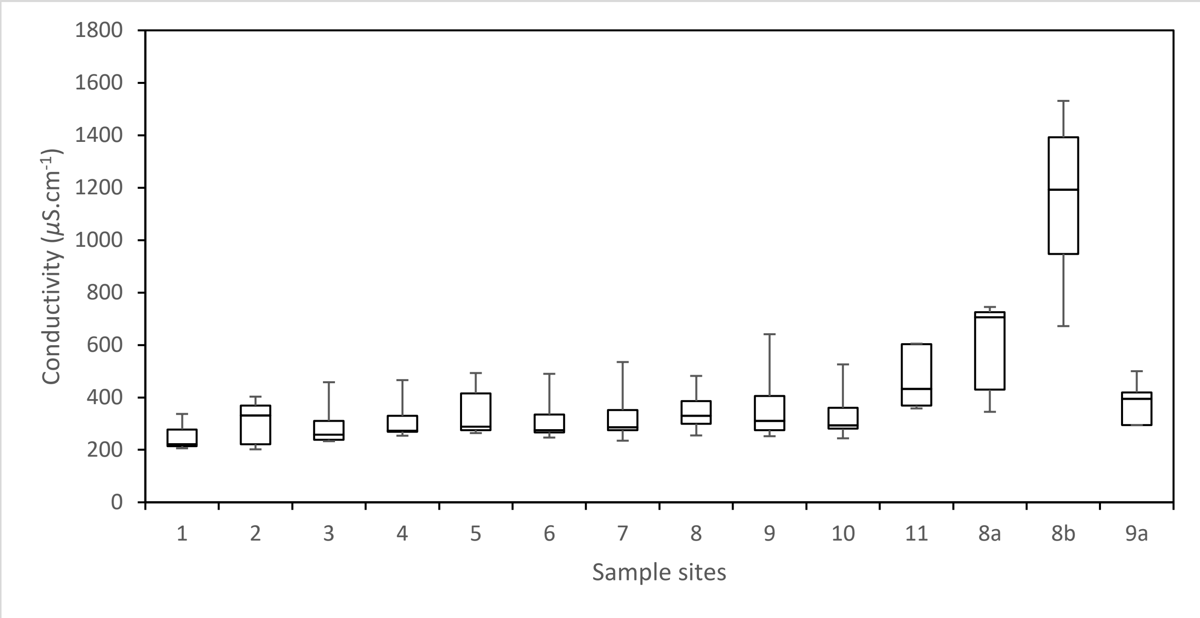

Figure 4.3: Box and whisker plot of conductivity (μS.cm^{-1}) collected during all field visits at sample sites going from upper stream to lower stream (sites 1 to 11), with the last three sites corresponding to side channels which are distinctly different from other sites (Table 3.1). The 'box' represents median and 25th/75th percentiles values and 'whiskers' representing maximum and minimum values.

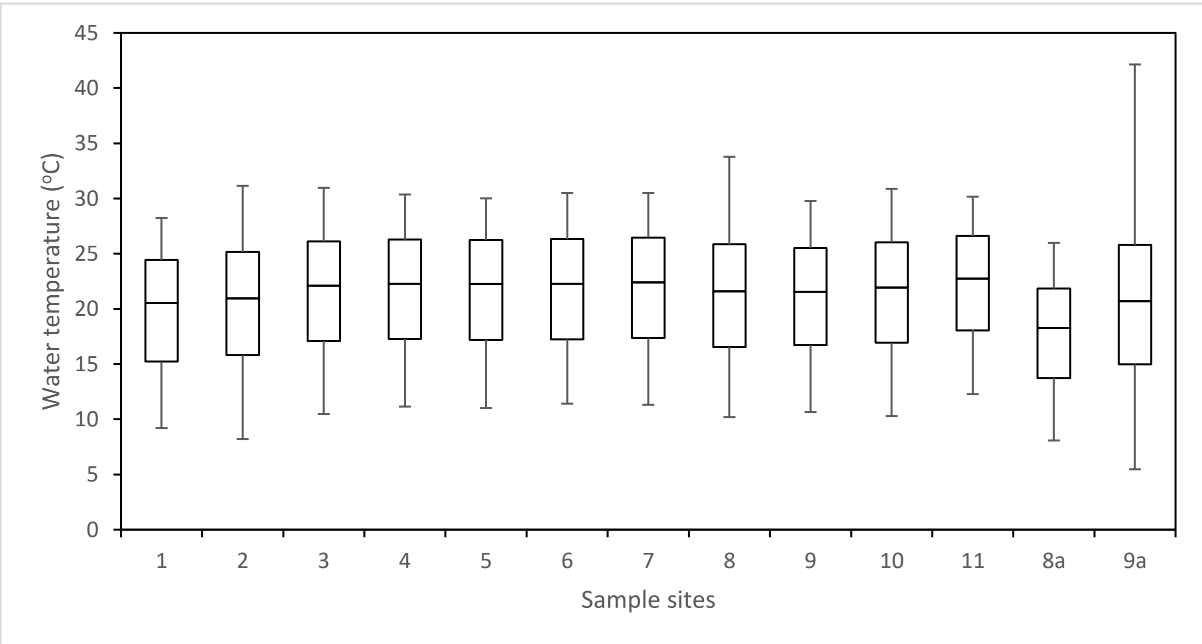

Figure 4.4: Box and whisker plot of hourly water temperatures collected during field visits at sample sites going from upper stream to lower stream (sites 1 to 11), with the last three sites corresponding to side channels which are distinctly different from other sites (Table 3.1). The 'box' represents median and 25th/75th percentiles values and 'whiskers' representing maximum and minimum values.

4.3. Blackfly species data

Collected blackfly showed a dominance by *S.chutteri* (in November 2015 and March 2016) and *S.damnosum* (in July 2016 and December 2016) (Figures 4.5a-d). November 2015 had three species, while the other sampling periods had six. *S.chutteri* showed a dominance in November 2015 (69% of the samples) (Figure 4.5a) and in March 2016 (70%) (Figure 4.5b). The next two sampling periods were predominantly *S.damnosum* (63%) in July 2016 (Figure 4.5c) and in December 2016 (54%) (Figure 4.5d).

At each site during each sampling period, the number of blackfly individuals found on substrata varied. Each sampling period showed that all species were not found at all sites, and some sites showed a dominance of one species whilst others showed more of a variation (Figure 4.6). *S.chutteri*, *S.damnosum* and *S.adersi* were found at the majority of the sampling sites whilst *S.macmahoni*, S.*impukane, S.ruficorne* and *S.nigritarse* were restricted to a few sites (Figure 4.6 to 4.9).

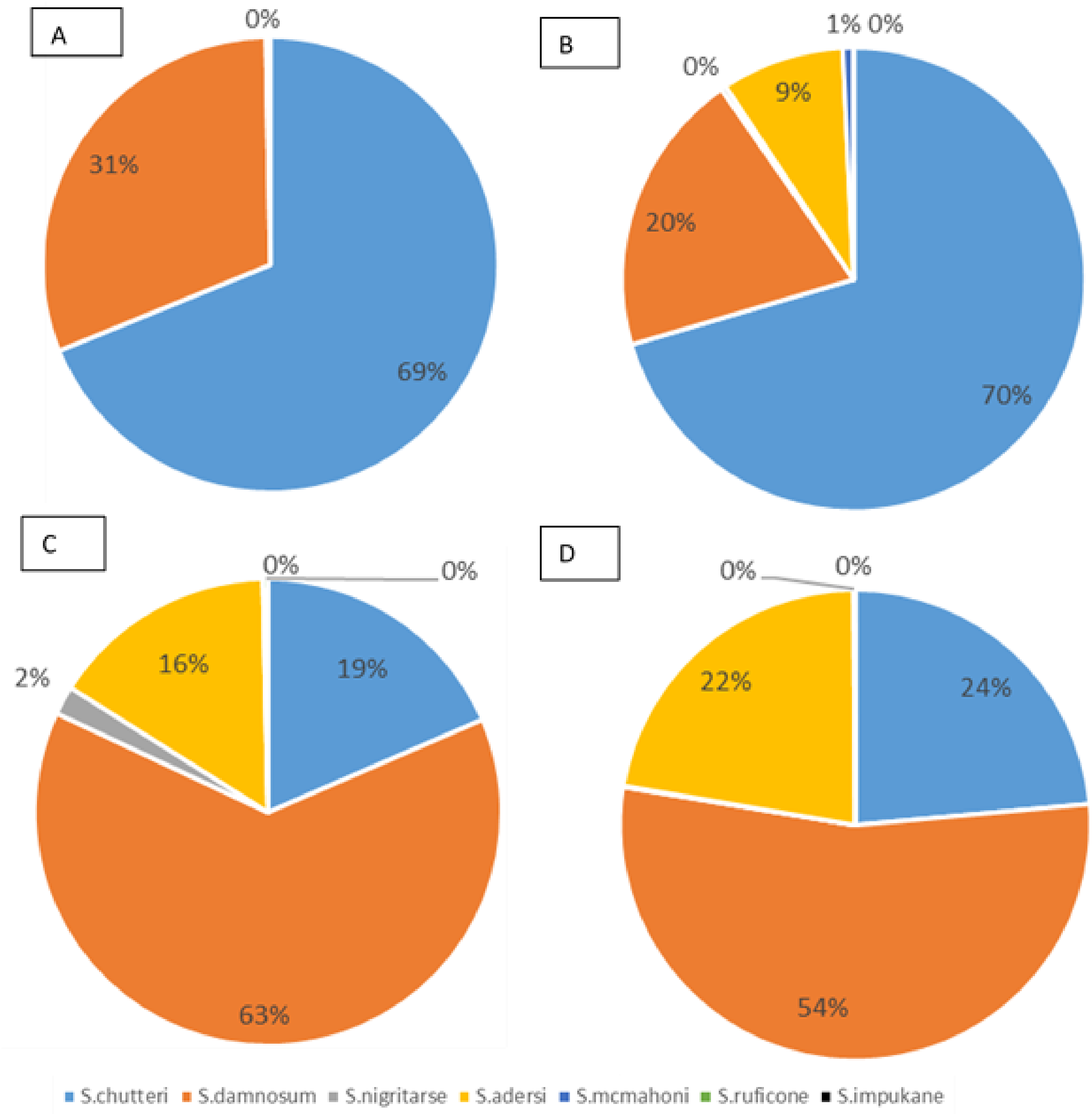

Figure 4.5: Combined percentage (%) of *Simulium* species: (a) November 2016; (b) March 2016; (c) July 2016; (d) December 2016.

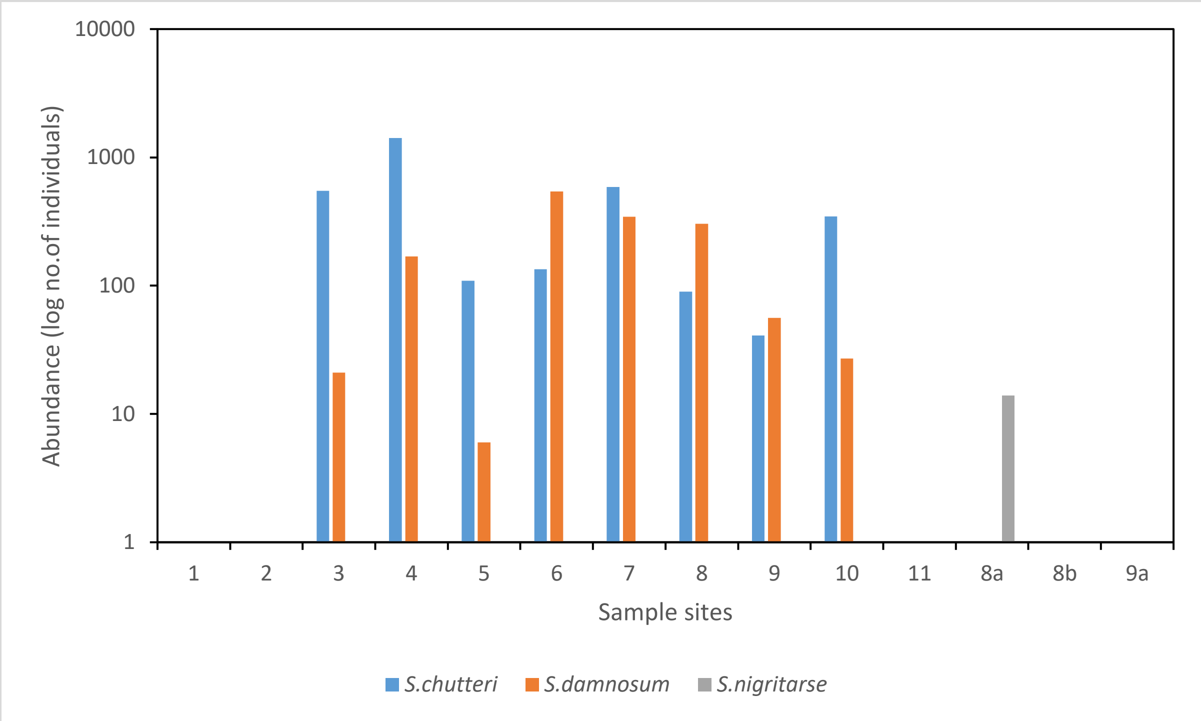

Figure 4.6: Semi-log plot of blackfly individuals found at each sampling site for November 2015, from upper stream to lower stream on the Orange River between sites 1 and 11. The last three sites correspond with side channels which are distinctly different from other sites, as per Table 3.1. Sites 1, 2 and 11 were not sampled during this period due to time limitations and site 8a and 9a had no species.

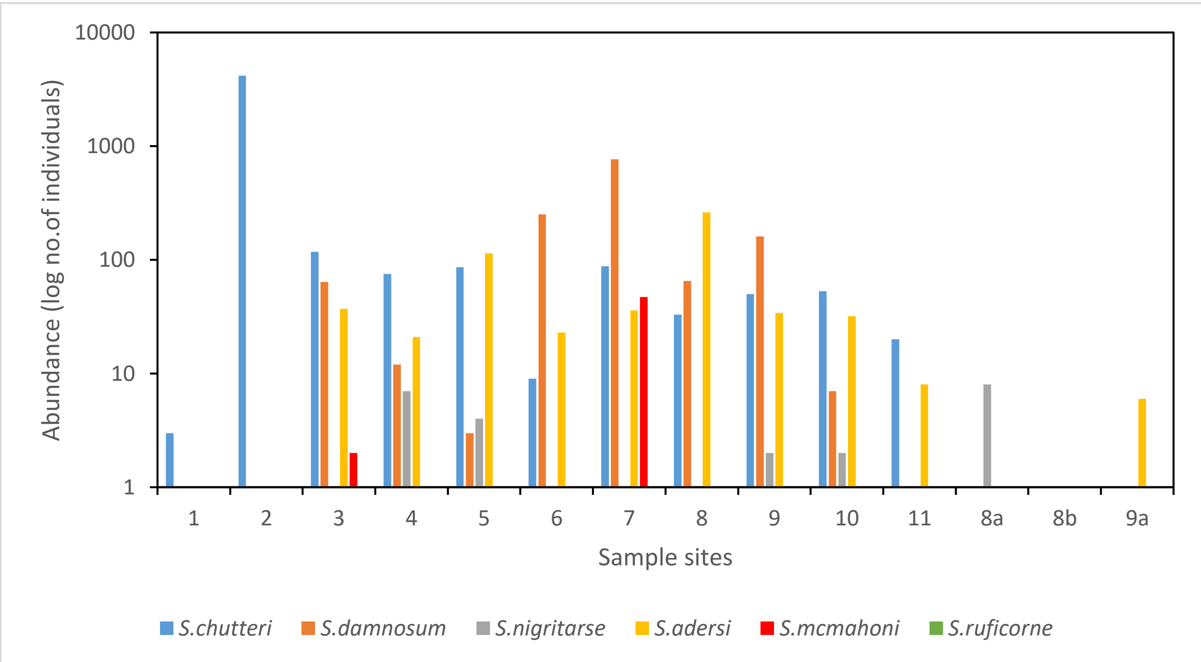

Figure 4.7: Semi-log plot of blackfly individuals found at each sampling site for March 2016, from upper stream to lower stream on the Orange River between Site 1 and 11. The last three sites correspond with side channels which are distinctly different from other sites, as per Table 3.1.

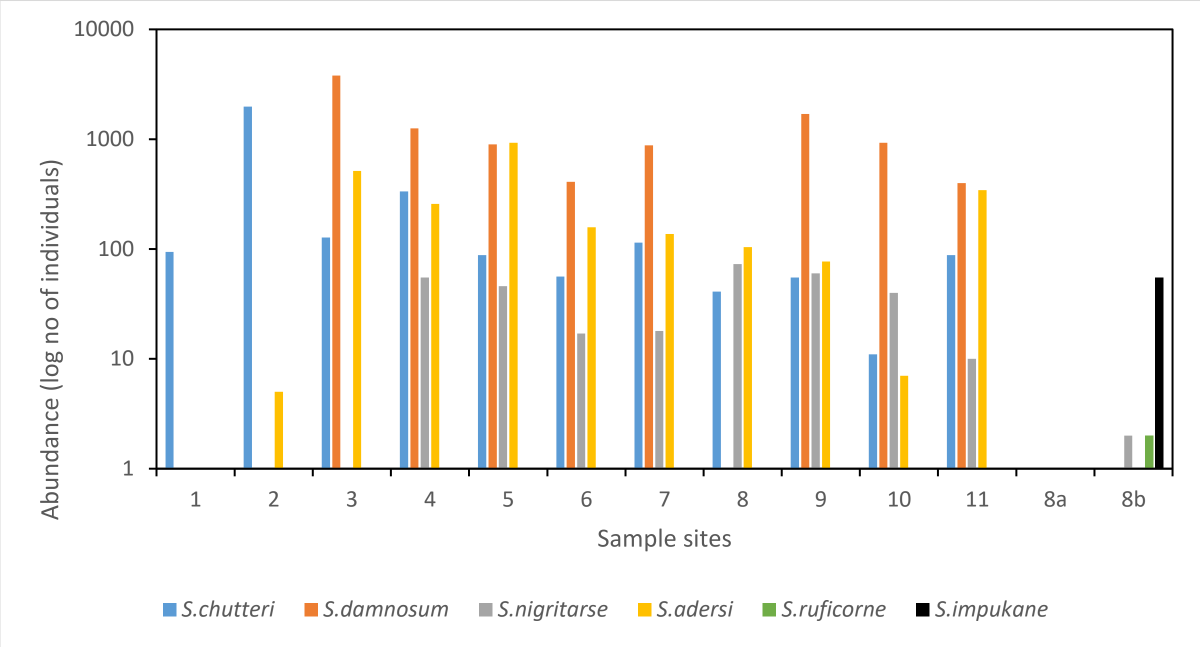

Figure 4.8: Semi-log plot of blackfly individuals found at each sampling site for July 2016, from upper stream to lower stream on the Orange River between Site 1 and 11. The last three sites correspond with side channels which are distinctly different from other sites, as per Table 3.1. Site 9a was dried up therefore no sampling was done during this site visit.

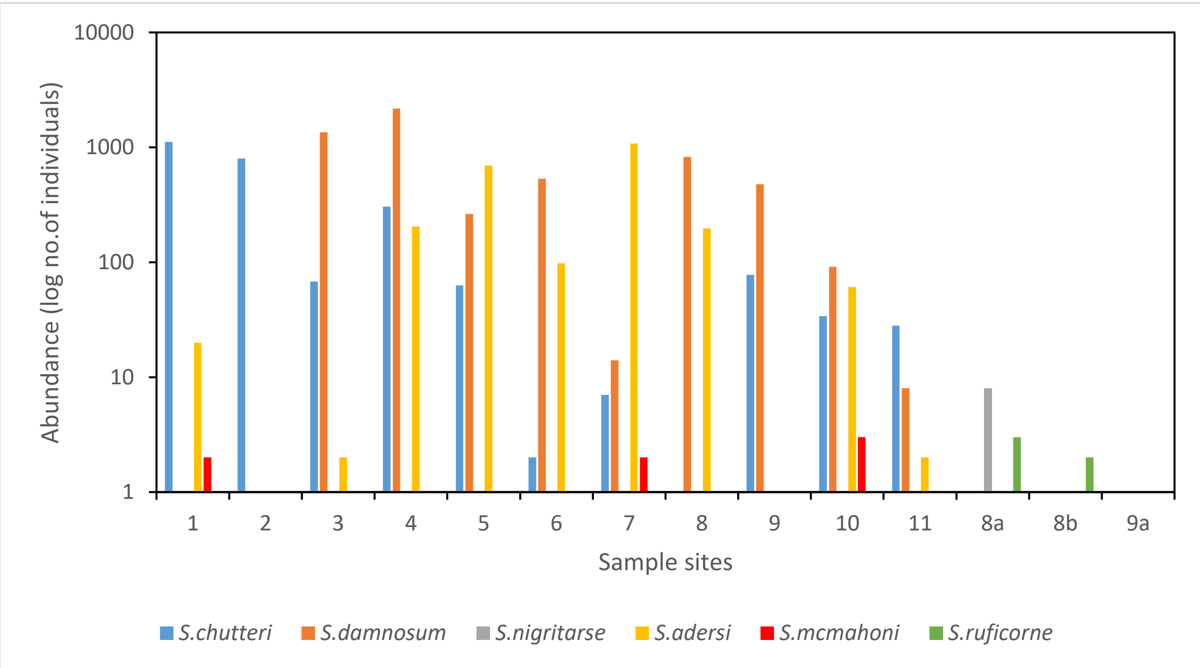

Figure 4.9: Semi-log plot of blackfly individuals found at each sampling site for December 2016, from upper stream to lower stream on the Orange River between Site 1 and 11. The last three sites correspond with side channels which are distinctly different from other sites, as per Table 3.1.

4.4. Species Thresholds

Blackfly species thresholds were identified and used in the Bayesian networks (Bns) to aid with state population of relevant nodes. Critical values were determined and were used as values that separate a node's state within the Bayesian networks (Bns), for which return intervals were calculated to provide probabilities of each state. Seven species of blackfly were collected for the sampling periods. Palmer and Craig (2000), show that these seven species can be grouped based on labral fan structure and preferences to flow velocities and seston concentrations (Figure 4.10). Three groups are defined from this model (Figure 4.10); strong porous, standard and weak complex group. Collected blackfly larvae and pupae showed that species found within the strong porous group (*S.chutteri* and *S.damnosum*) and species found in the standard group (*S.adersi*) are the species likely to cause an outbreak. Species found in the weak complex group were not found regularly and therefore not considered as a group that could cause an outbreak. Therefore, the Bns would only include the strong porous and standard complex groups. Palmer and Craig's (2000) model was used along with the thresholds determined to obtain critical values for the nodes used within the Bns.

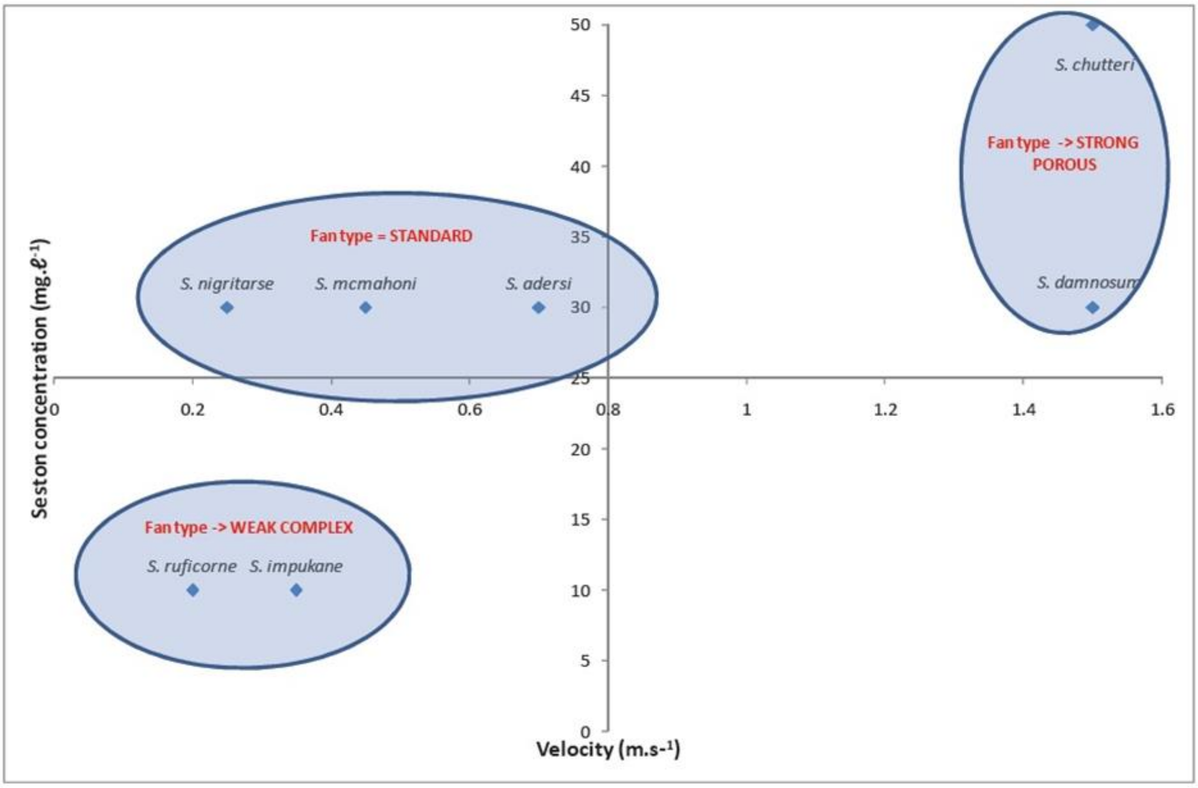

Figure 4.10: Conceptual grouping of Orange River blackfly species, based on labral fan types with determine seston concentration and velocity preferences (Palmer and Craig, 2000, Rivers-Moore and Palmer, 2017).

4.4.1. Flow velocity thresholds

Species thresholds for flow velocity from Palmer and Craig's (2000) model illustrates that for the strong porous group, flow velocities between 1 $m.s^{-1}$ to 2 $m.s^{-1}$ are generally preferred (Figure 4.10 and Table 4.2). From the data collected from November 2015 to December 2016, flow velocities for species in the strong porous group (*S.chutteri* and *S.damnosum)* support Palmer and Craig's (2000) model, as it showed an increase in species abundance with velocity, indicating that this group prefers high velocities (Figure 4.11). For the standard group, Palmer and Craig's (2000) model shows that the species within this group prefers velocities in the range of 0.2 $m.s^{-1}$ to 0.9 $m.s^{-1}$ (Figure 4.10 and Table 4.2). From the data collected from November 2015 to December 2016, *S.adersi* shows a peak at approximately 0.8 $m.s^{-1}$ which is supportive of Palmer and Craig's (2000) model, whilst *S.nigritarse* and *S.mcmahoni* (which belong to the standard complex group) did not show any distinctive preferences to flow velocities (Figure 4.11). From thresholds determined by the data collected, aided with Palmer and Craig's (2000) model, a flow velocity of 0.8 $m.s^{-1}$ was identified as the critical value. This value was not based on the threshold value observed for *S.adersi*, but rather a conservative value for *S.chutteri* and *S.damnosum* due to these species being the most abundant found during sampling periods. Values above 0.8 $m.s^{-1}$ would be considered high velocities for which *S.chutteri* and *S.damnosum* are more than likely to be found, whereas values less than 0.8 $m.s^{-1}$ would be considered low velocities for which other species are likely to be found. However, with the node within the Bns not being flow velocity ($m.s^{-1}$), but rather discharge ($m^3.s^{-1}$) (Refer to section 3.4.3.1), the critical value 0.8 $m.s^{-1}$ was converted to 65 $m^3.s^{-1}$ (Palmer, 1997). Therefore <65 $m^3.s^{-1}$ would be considered low discharge and >65 $m^3.s^{-1}$ would be considered high discharge.

4.4.2. Seston concentration thresholds

Species thresholds for seston concentrations ($mg.\ell^{-1}$) from Palmer and Craig's (2000) model shows that for the strong porous group, seston concentrations between 30 $mg.\ell^{-1}$ to 50 $mg.\ell^{-1}$ are generally preferred (Figure 4.10 and Table 4.3). From the data collected from November 2015 to December 2016, species from the strong porous group showed a wider range than the Palmer and Craig's (2000) model. The species in this group showed high abundances in seston concentrations of 10 to >50 $mg.\ell^{-1}$, with the highest concentration found between 20 to 30 $mg.\ell^{-1}$ (Figure 4.12 and Table 4.3). For the standard group, Palmer and Craig's (2000) model shows that the species within this group prefers seston concentrations in the range of approximately 20 to 40 $mg.\ell^{-1}$ (Figure 4.10). From the data collected from November 2015 to December 2016, *S.adersi* showed similar threshold ranges as the species in the strong porous group of 10 to >50 $mg.\ell^{-1}$ (Table 4.3), with highest species abundance concentration found at 20 $mg.\ell^{-1}$ (Figure 4.12). Another species belonging to the standard group, *S.nigritarse*, showed a range from 6 to 30 $mg.\ell^{-1}$, with the highest concentrations being found at 20 $mg.\ell^{-1}$ (Figure 4.12). Thresholds determined by the data collected aided by Palmer and Craig's (2000) model, a critical value of 25 $mg.\ell^{-1}$ could be used as the value to separate node states within the Bayesian networks (Bns). Therefore <25 $mg.\ell^{-1}$ would be considered low seston concentration and >25 $mg.\ell^{-1}$ would be considered high seston concentration.

4.4.3. pH and Conductivity thresholds

These two variables were excluded from the Bayesian networks (Bns) as there are no distinct thresholds for the strong porous and standard groups (Figure 4.13 and 4.14). For pH, species did not show any preference to pH levels (4.13). For conductivity ($\mu S.cm^{-1}$), there was a clear threshold observed for the weak porous complex group, as it was seen that they prefer highly conductive waters of >700 $\mu S.cm^{-1}$ (Figure 4.14). However, the strong porous and standard group did not show any apparent thresholds or preferences (Figure 4.14) which is why this variable was excluded from the Bns.

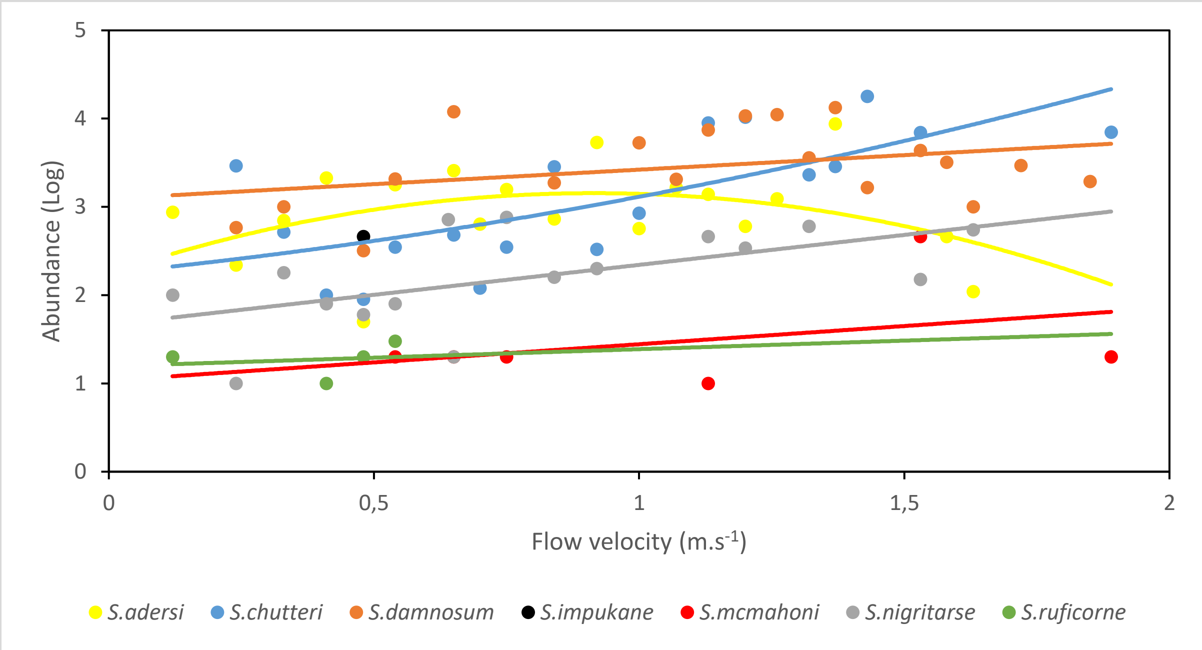

Figure 4.11: Log-transformed abundances of seven species of blackfly using combined sample data from November 2015 to December 2016 for maximum abundances at each velocity value. 2nd order polynomials were used to describe the relationship between flow velocity ($m.s^{-1}$) and log abundance for all species.

Table 4.1: Equations describing relationship between flow velocity and abundance for six species of *Simulium* (see Figure 4.8a). *S.impukane* was excluded as this species was only found once and at one flow velocity.

Species	Equation	R^2
S.adersi	$y = -1{,}0875x^2 + 1{,}989x + 2{,}246$	0,1584
S.chutteri	$y = 0{,}2673x^2 + 0{,}5973x + 2{,}2496$	0,5058
S.damnosum	$y = 0{,}329x + 3{,}093$	0,1212
S.mcmahoni	$y = 0{,}6786x + 1{,}6646$	0,3219
S.nigritarse	$y = 0{,}412x + 1{,}0322$	0,1397
S.ruficorne	$y = 0{,}1933x + 1{,}1949$	0,0330

Table 4.2: Blackfly species optimal flow velocity ($m.s^{-1}$) thresholds determined by Palmer and Craig (2000) and from collected field data.

Species	Palmer and Craig (2000)	Collected field data
S.chutteri	1.4 – 1.6 $m.s^{-1}$	1 – 1.8 $m.s^{-1}$
S.damnosum	1.4 – 1.6 $m.s^{-1}$	0.7 – 1.8 $m.s^{-1}$
S.adersi	0.6 – 0.8 $m.s^{-1}$	0.6 – 1 $m.s^{-1}$
S.macmahoni	0.4 – 0.6 $m.s^{-1}$	Not distinct
S.nigritarse	0.2 – 0.4 $m.s^{-1}$	Not distinct
S.ruficorne	0 – 0.2 $m.s^{-1}$	Not distinct
S.impukane	0.2 – 0.4 $m.s^{-1}$	Not distinct

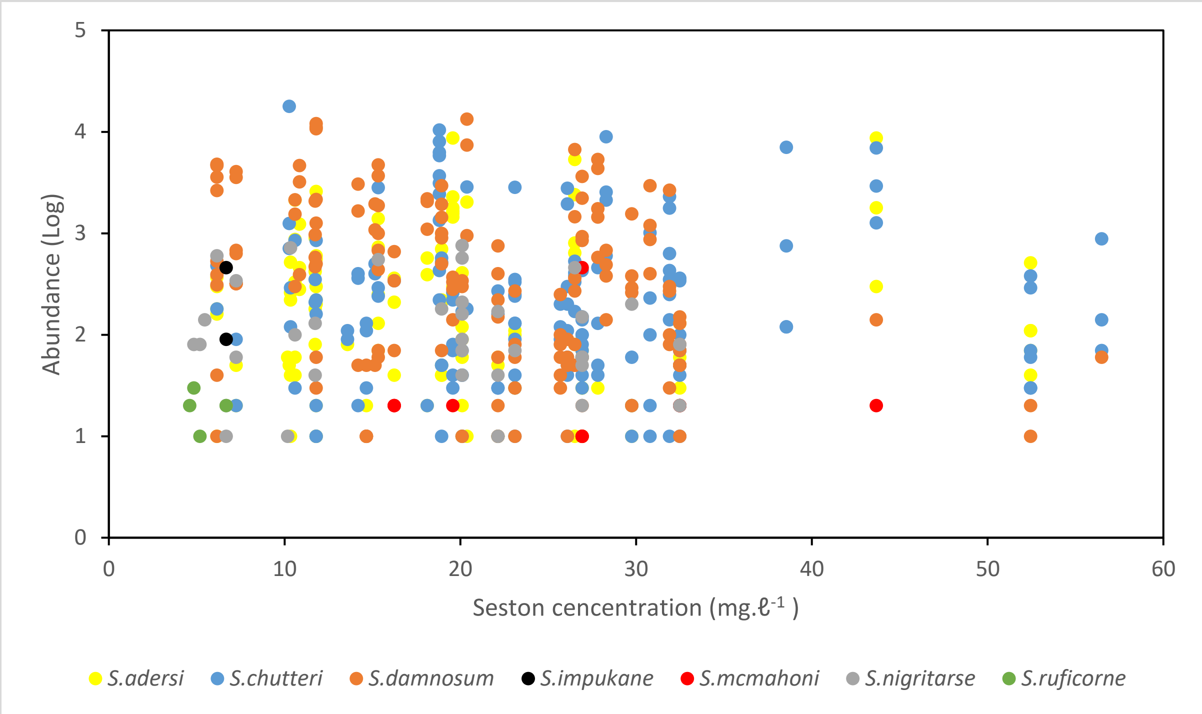

Figure 4.12: Log-transformed abundances of seven species of blackfly based on combined sample data from November 2015 to December 2016 against seston concentration (mg.ℓ$^{-1}$).

Table 4.3: Blackfly species optimal seston concentration (mg.ℓ$^{-1}$) thresholds determined by Palmer and Craig (2000) and from collected field data.

Species	Palmer and Craig (2000)	Collected field data
S.chutteri	50+ mg.ℓ$^{-1}$	25 – 55 mg.ℓ$^{-1}$
S.damnosum	±30 mg.ℓ$^{-1}$	10 – 30 mg.ℓ$^{-1}$
S.adersi	±30 mg.ℓ$^{-1}$	10 – 50 mg.ℓ$^{-1}$
S.macmahoni	±30 mg.ℓ$^{-1}$	20 – 30 mg.ℓ$^{-1}$
S.nigritarse	±30 mg.ℓ$^{-1}$	6 – 30 mg.ℓ$^{-1}$
S.ruficorne	±10 mg.ℓ$^{-1}$	0 – 10 mg.ℓ$^{-1}$
S.impukane	±10 mg.ℓ$^{-1}$	0 – 10 mg.ℓ$^{-1}$

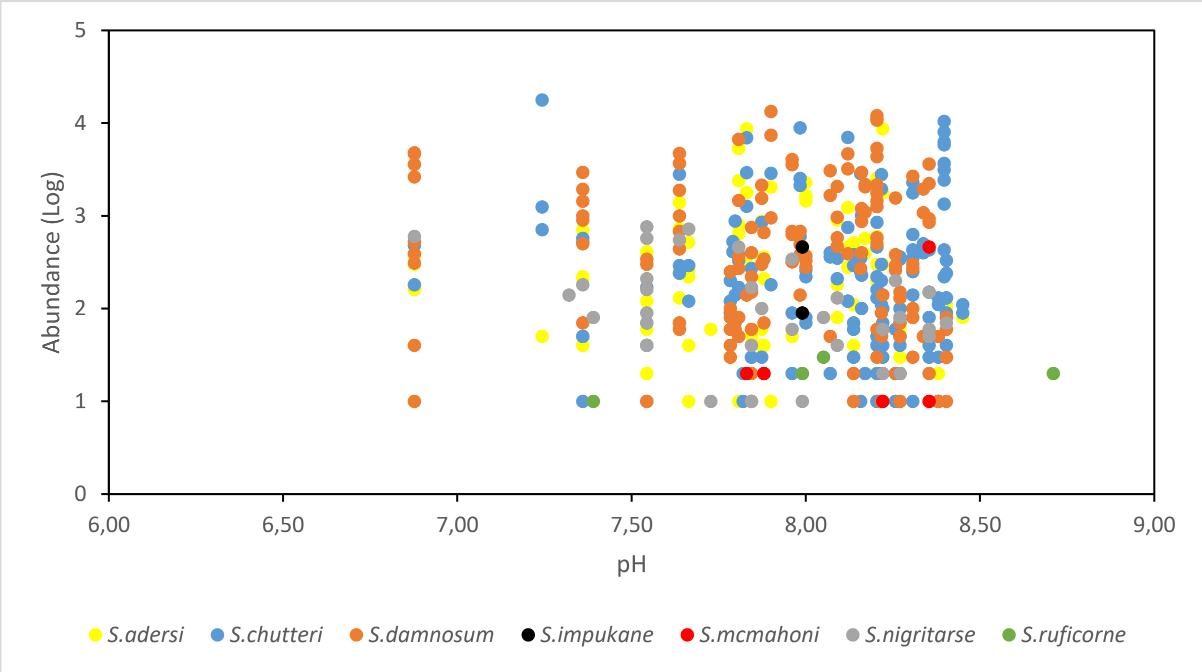

Figure 4.13: Log-transformed abundances of seven species of blackfly based on combined sample data from November 2015 to December 2016 against pH.

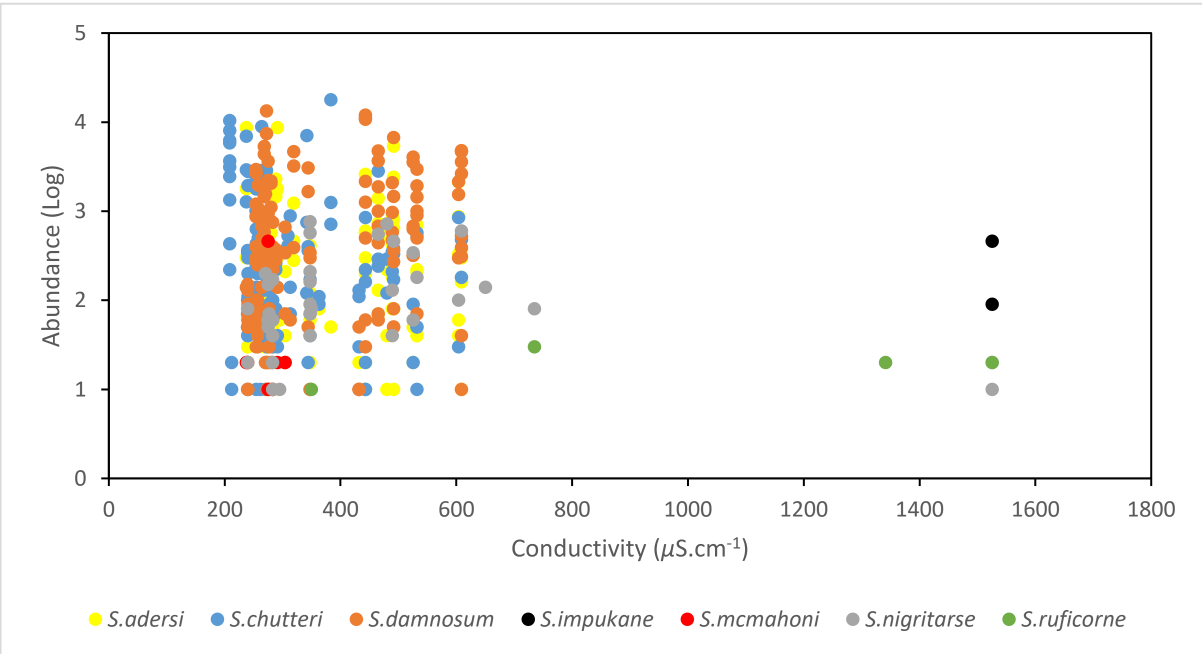

Figure 4.14: Log-transformed abundances of seven species of blackfly based on combined sample data from November 2015 to December 2016 against conductivity ($\mu S.cm^{-1}$).

4.5. Bayesian Network Models

4.5.1. Spatial and Temporal resolution of the Bayesian Networks

A simple Bayesian Network (Bn) was developed based on the Bn of Rivers-Moore *et al.,* (2014). Variables used within the Bns were known to affect blackfly outbreaks along the Orange River (Table 3.3). For the spatial resolution of the Bns, Principal Components Analysis (PCAs) and cluster analysis were used to group sites to avoid a high number of unnecessary Bns. The first analysis was run against seston concentration, pH, conductivity and seasonal variations (Figure 4.15 and 4.16). Based on PCAs and cluster analysis of these variables, there was little evidence to support clear grouping of sites, but rather a trend supporting seasonal variation of water quality (Figure 4.15 and 4.16). The second analysis run was against daily mean water temperatures, which were derived from metrics using the hourly water temperatures from the HOBO loggers installed at each site. This analysis showed two clear groupings of sites with a third outlier group consisting of a single site (Figure 4.17 and 4.18). The first grouping consists of sites 1,2 and 9a (Refer to Table 3.1). The second grouping consists of sites 3,4,5,6,7,8,9,10 and 11, with the final grouping consisting of site 8a (Refer to Table 3.1).

Therefore, spatially there were two models developed from the sample sites, as a model for site 8a would not be a viable option. The groupings were distinctive of a geographic/altitudinal split, except for site 9a being grouped with sites 1 and 2. Therefore, the first Bn was called upper stream sites, and the second Bn was called lower stream sites. Temporally, each model was initially run 12 times (section 3.4.1) to generate blackfly outbreak probabilities for each month of the year, and thereafter refined to seasonal outbreak probabilities.

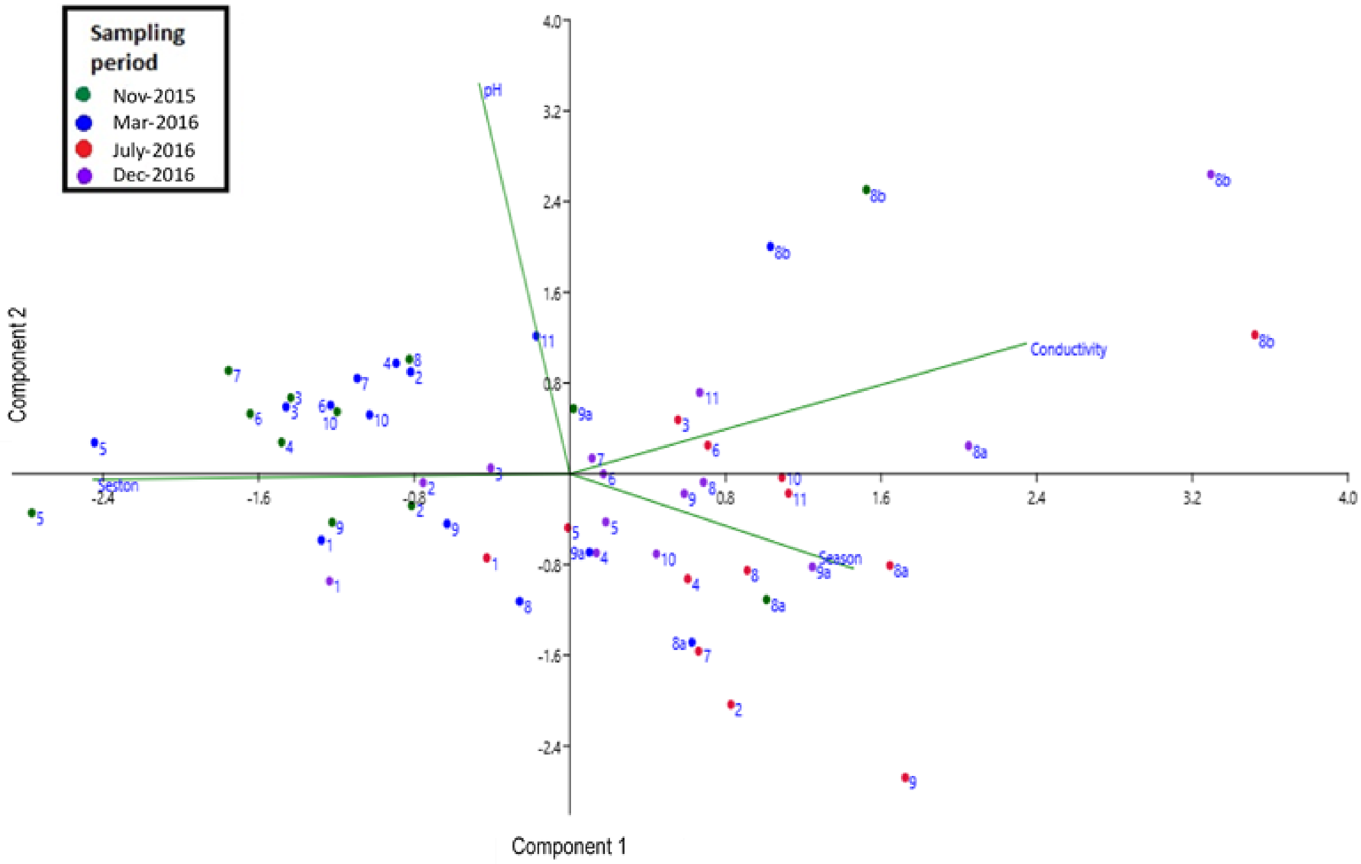

Figure 4.15: Principal Components Analysis (PCA) of study sites for water quality for the four sampling periods.

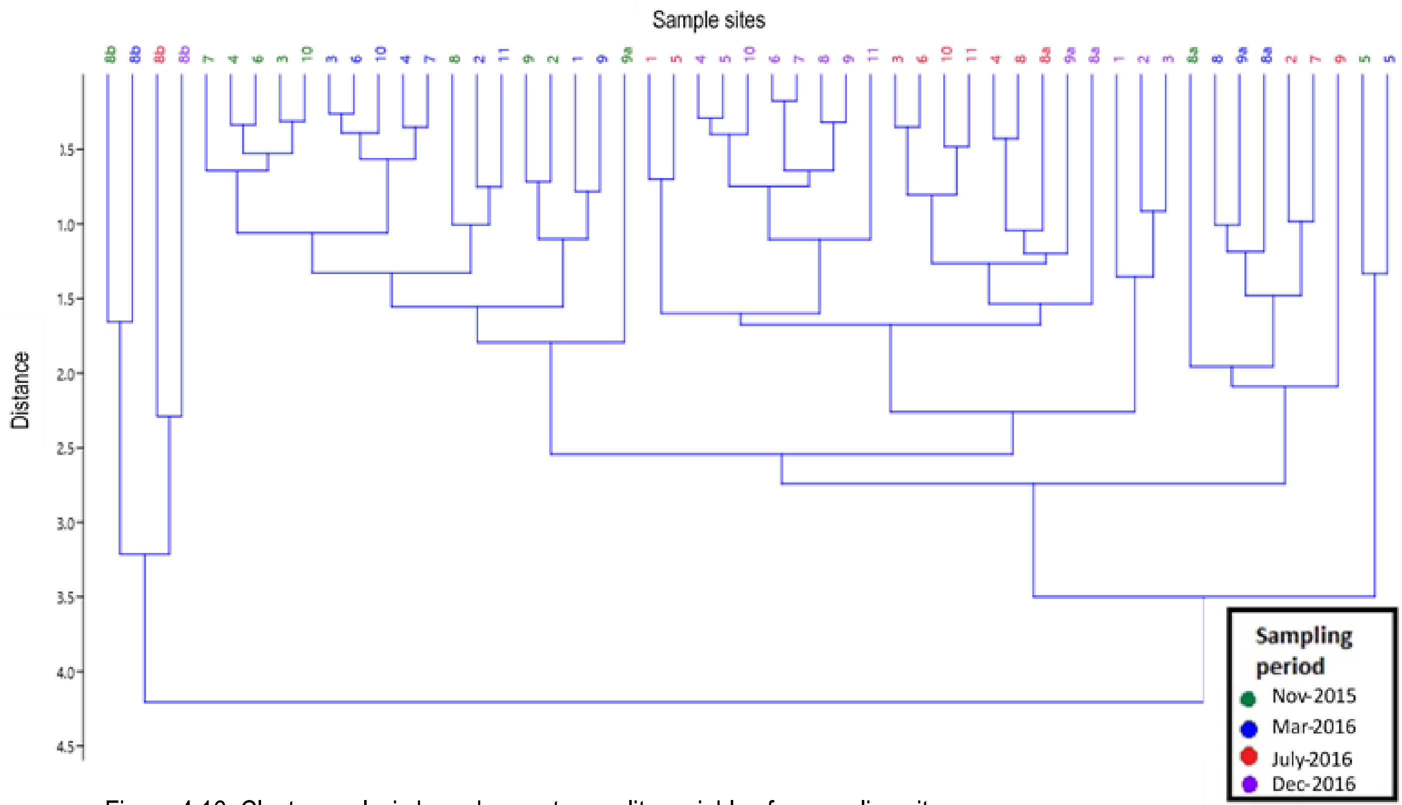

Figure 4.16: Cluster analysis based on water quality variables for sampling sites

Table 4.4: Eigenvalues and cumulative percentage variance for water quality variables for study sites.

	PC Component 1	**PC Component 2**	**PC Component 3**	**PC Component 4**
Cumulative % variance	41.6%	25.8%	21.8%	10.9%
Variables		**Eigenvalues**		
Seston	-0.66	-0.01	0.27	0.70
pH	-0.13	0.92	0.29	-0.21
Conductivity	0.63	0.31	-0.21	0.68
Season	0.39	-0.22	0.89	0.02

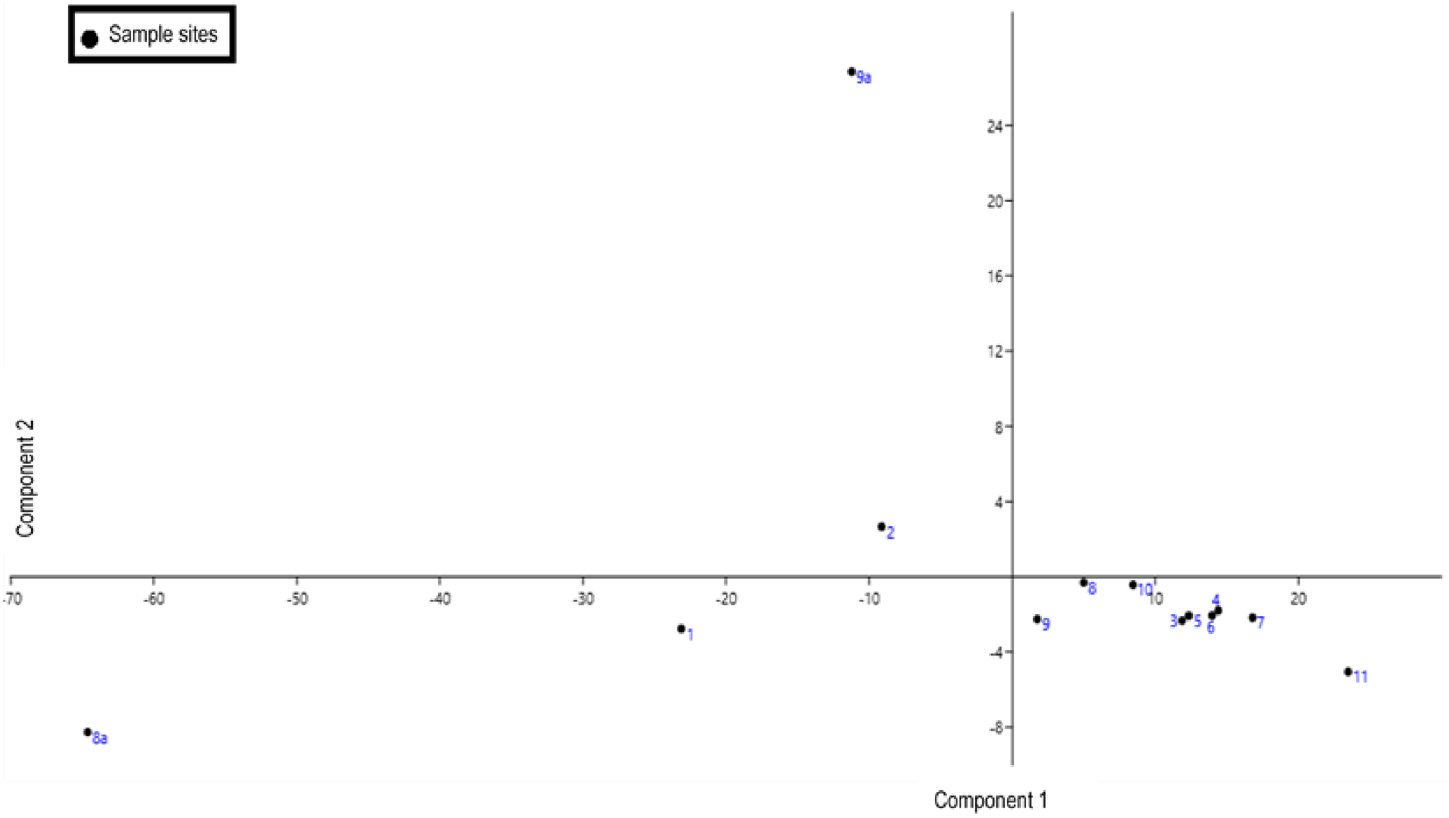

Figure 4.17: Principal Components Analysis (PCA) of study sites based on daily mean water temperature derived from metrics. Cumulative % variance of PC components 1 and 2 was 84% and 11% respectively.

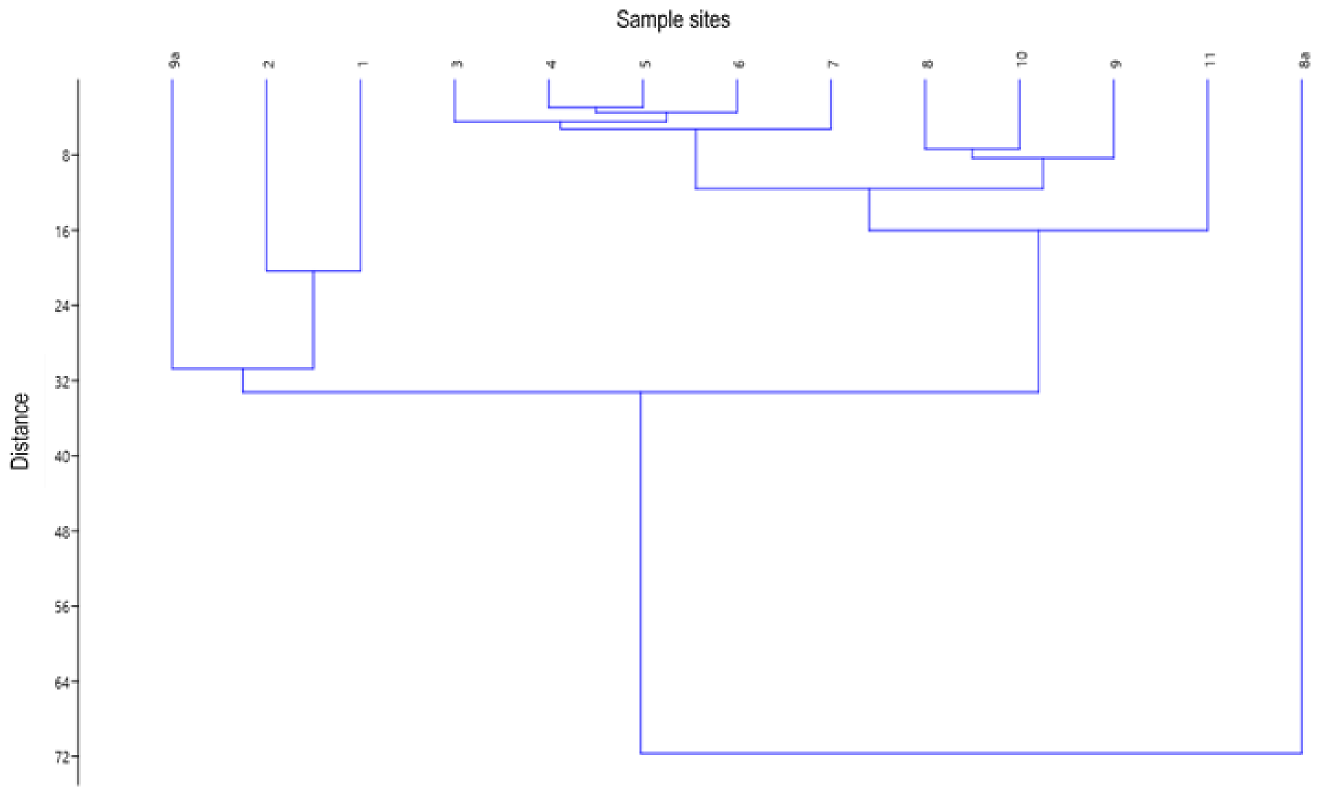

Figure 4.18: Cluster analysis based on mean daily water temperatures derived from metrics.

4.5.2. *Node probability population of 'parent' nodes in the Bayesian networks (Bns)*

Within the Bayesian networks (Bns), the 'parent' nodes were; discharge, seston concentration, water temperature, channel type, reeds and spraying. 'Parent' node probabilities for their states were calculated for each spatial model (Table 4.5 and 4.6).

4.5.2.1. Discharge ($m^3.s^{-1}$)

For discharge state probabilities calculation of the upper stream model, the dataset used for return interval calculation was from the Prieska gauging weir (D7H002) (Figure 4.19), as this weir was geographically close and had long-term data for sites 1 and 2 (Table 3.3). For the lower stream model, the dataset used was from the Upington weir (D7H005) (Figure 4.20) as this weir was geographically close and had long-term data for sites 3-11 (Table 3.3). Return intervals were calculated (See section 3.4.3) for flows below or exceeding 65 $m^3.s^{-1}$ (which was the critical value obtained from section 4.4.1) for the weirs used in each model (Figure 4.21). Return interval calculation of each weir showed the same probabilities percentage below and exceeding the critical value of 65 $m^3.s^{-1}$, therefore, discharge node states had the same probabilities for both models (Table 4.5a-b).

4.5.2.2. Seston concentration *($mg.\ell^{-1}$)*

As there was no time series data for seston concentration ($mg.\ell^{-1}$), ideally, modelled values of seston concentration from discharge could be used to obtain an accurate time series dataset. As was the case for discharge state probabilities, the same gauging weirs were used for both models. Discharge values were converted to seston concentration ($mg.\ell^{-1}$) using equation 2 (section 3.4.3.2). These values were plotted against converted seston concentration ($mg.\ell^{-1}$) data (from water clarity using equation 1, section 3.3.1.2) over time (Figure 4.22 and 4.23). A poor relationship was observed with each other, with a R^2 value of 0.1033 for data relative to gauging weir D7H002 (Upper stream model) and a R^2 value of 0.0773 for data relative to gauging weir D7H005 (Lower stream model) (Figure 4.24 and 4.25). This indicates a poor correlation between seston concentration obtained from discharge and water clarity readings. Data collected and converted from DAFF monitoring showed a similarly poor correlation between converted seston concentrations from discharge and water clarity (Figure 4.26, 4.27 and 4.28). This indicates that seston concentrations obtained from discharge data from gauging weirs are not accurate enough to use when calculating probabilities for states within the Bns. Seston concentration data converted from water clarity readings collected during field visits and weekly data collected by DAFF during their monitoring were combined to calculate return intervals (See section 3.4.3). Although this dataset were not long-term, there was confidence in the accuracy of the data which was more desirable compared to a long-term dataset that shows no-relationship with other seston concentrations. State probabilities for the node seston concentration were calculated from return intervals for seston concentrations below or exceeding the critical value of 25 $mg.\ell^{-1}$ (section 4.4.2) within the each Bns (Figure 4.29). The upper stream model showed a higher probability of seston concentrations exceeding the critical value than the lower stream model (Table 4.5 and 4.6).

4.5.2.3. Water temperature

For the upper stream model, the site closest to gauging weir D7H002 was site 2, and therefore the water temperatures from this site was used to calculate state probabilities (Figure 4.30). For the lower stream model, the site closest to weir D7H005 was site 5, and therefore the water temperatures from this site was used to calculate state probabilities (Figure 4.30). A critical value of 20° C (an assumed trade-off value from the literature (Molloy and Jamnback., 1981; Begemann, 1986; Palmer, 1997; Paerl and Huisman, 2008)) was used and return intervals calculated (See section 3.4.3) for data below or exceeding this value for each Bn (Table 4.5 and 4.6). The lower stream model showed a higher probability of values exceeding the critical threshold of 20° C than the upper stream model (Figure 4.31).

4.5.2.4. Channel type

Google earth was used to calculate the percentage of anastomosing segments of river between site 1 and site 11 using Equation 3 (section 3.4.3.4). Google earth coordinates of anastomosing segments along the Orange were given to Rivers-Moore and Palmer (2017), and plotted based on altitude and downstream distance from the Vanderkloof dam (Figure 4.32). Varying geologies of sites along the middle and lower reaches of the Orange River resulted in different state probabilities for each Bn (Table 4.5 and 4.6). The upper stream model had higher probabilities of the 'single' channel types than found for the lower 'stream' model, which had higher probabilities for 'anastomosing' channel types (Figure 4.32).

4.5.2.5. Reeds and Spraying

For the reed and spraying nodes in the Bns, there was no quantitative data available, therefore probabilities were obtained after case files were inputted and CPTs generated within each Bn (Table 4.5 and 4.6).

Table 4.5: Nodes, states and probabilities of the Bayesian network (Bn) for the upper stream model for blackfly outbreaks along the Orange River.

Nodes	States	Probabilities (%)
Discharge	Low/High	34% / 66%
Seston concentrations	Low/High	44% / 56%
Water temperature	Cool/Warm	36% / 64%
Channel type	Anastomosing/Single	10% / 90%
Reeds	Absent/Present	20% / 80%
Spraying	Unsuccessful/Successful	60% / 40%
Algae	Absent/Present	See CPT Table 4.7a
Larvicide efficacy	Suboptimal/Optimal	See CPT Table 4.8a
Abiotic	Unfavourable/Favourable	See CPT Table 4.9a
Biotic	Weak/Strong	See CPT Table 4.10a
Simulium group complex	Standard/Strong porous	See CPT Table 4.11a
Management	Effective/Poor	See CPT Table 4.12a
Outbreak Probabilities	Low/High	See CPT Table 4.13a

Table 4.6: Nodes, states and probabilities of the Bayesian network (Bn) for the lower stream model for blackfly outbreaks along the Orange River.

Nodes	States	Probabilities
Discharge	Low/High	34% / 66%
Seston concentrations	Low/High	54% / 46%
Water temperature	Cool/Warm	30% / 70%
Channel type	Anastomosing/Single	30% / 70%
Reeds	Absent/Present	20% / 80%
Spraying	Unsuccessful/Successful	47% / 53%
Algal blooms	Absent/Present	See CPT Table 4.7b
Larvicide efficacy	Suboptimal/Optimal	See CPT Table 4.8b
Abiotic	Unfavourable/Favourable	See CPT Table 4.9b
Biotic	Weak/Strong	See CPT Table 4.10b
Simulium group complex	Standard/Strong porous	See CPT Table 4.11b
Management	Effective/Poor	See CPT Table 4.12b
Outbreak Probabilities	Low/High	See CPT Table 4.13b

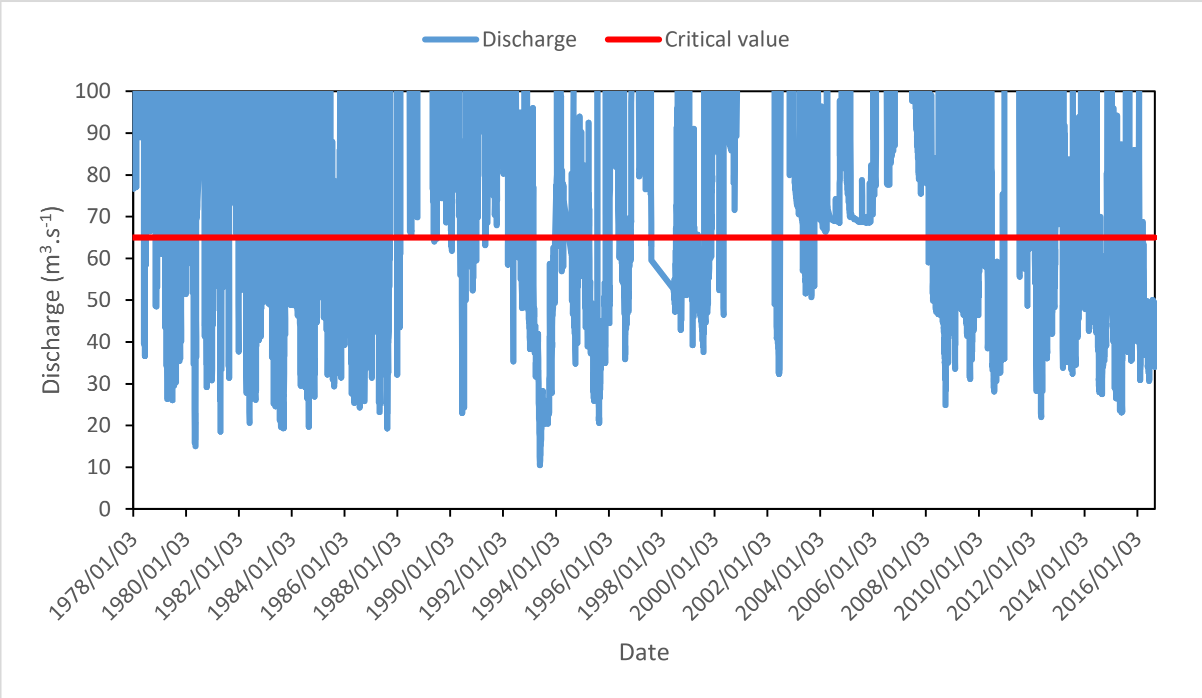

Figure 4.19: Post-impoundment time series discharge data from gauging weir D7H002 (upper stream model) used to calculate return intervals of values below or exceeding 65 $m^3.s^{-1}$.

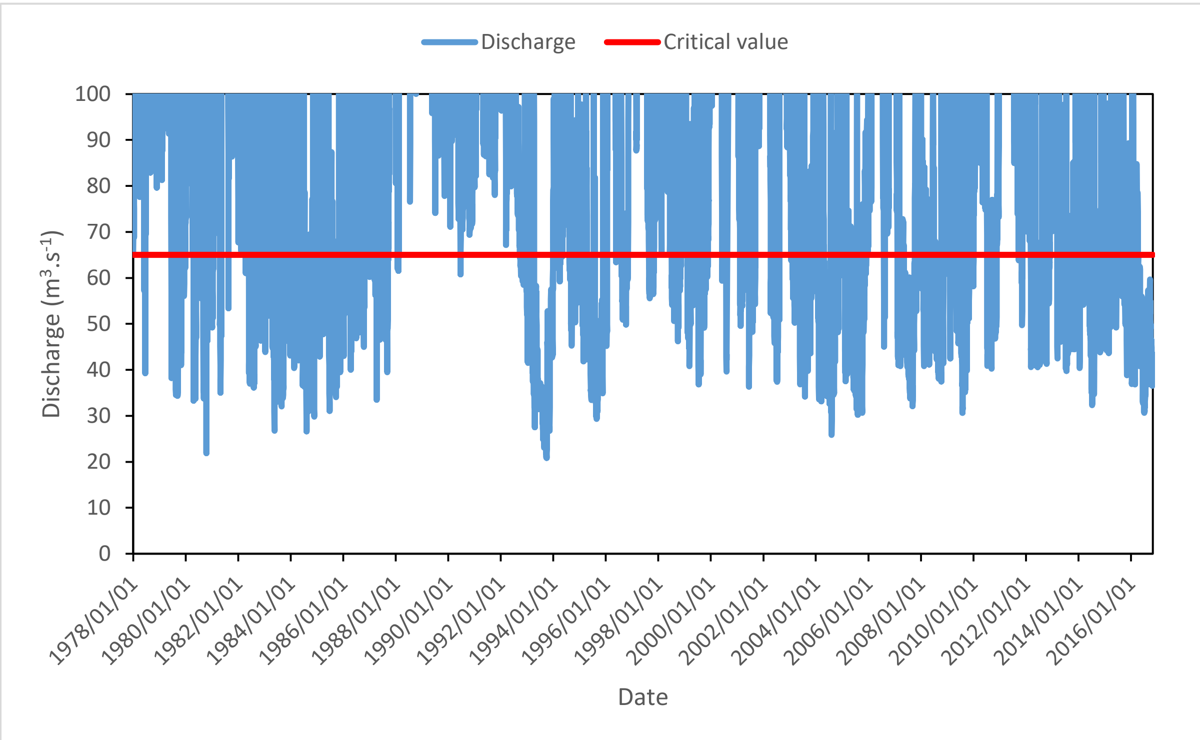

Figure 4.20: Post-impoundment time series discharge data from gauging weir D7H005 (lower stream model) used to calculate return intervals of values below or exceeding 65 $m^3.s^{-1}$.

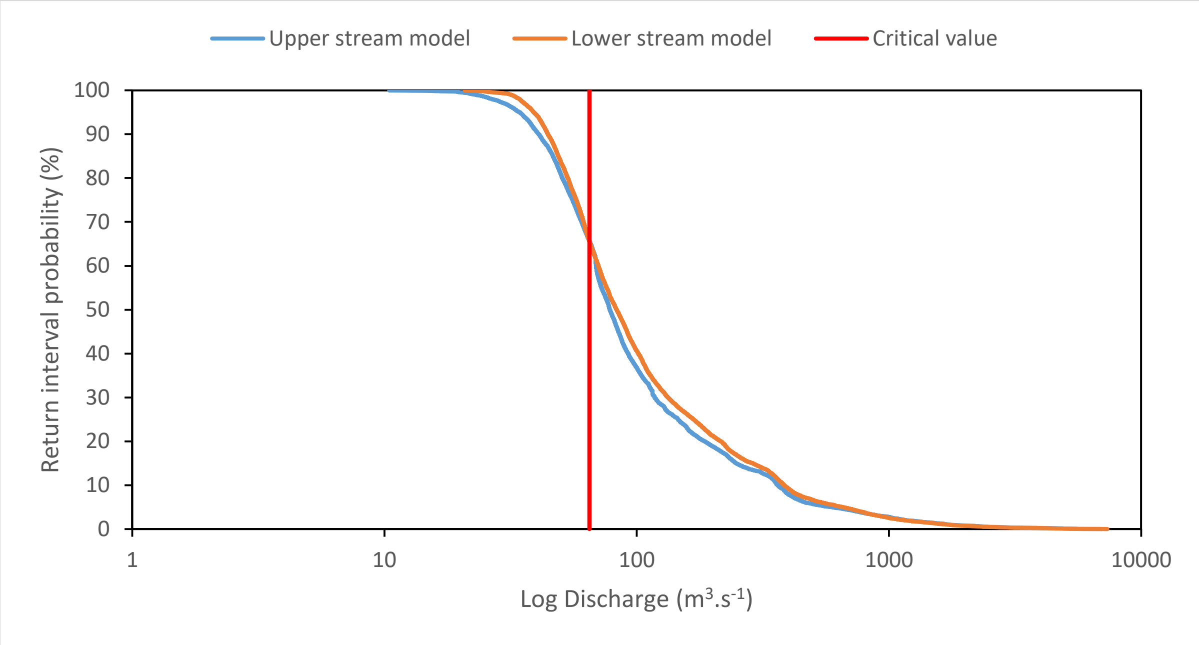

Figure 4.21: Discharge return interval calculation from gauging weir data for node probability calculation for Bayesian network models (Bns).

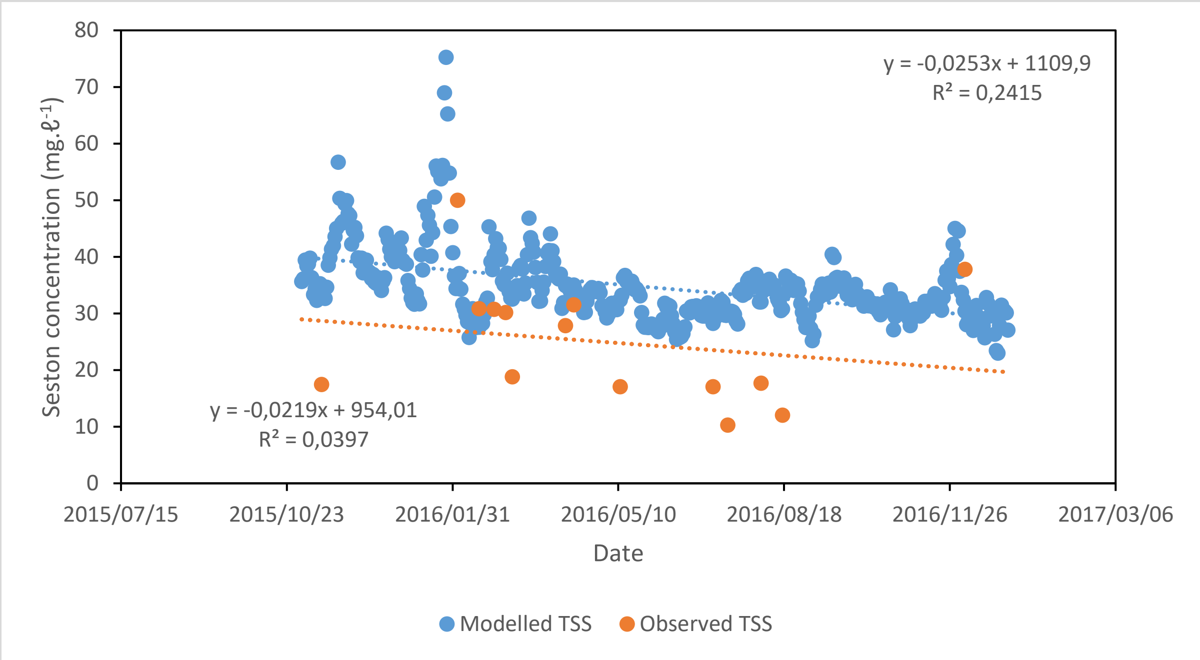

Figure 4.22: Modelled seston concentration (mg.ℓ$^{-1}$) over time from discharge data from Prieska weir D7H002, plotted with observed seston concentration (mg.ℓ$^{-1}$) data collected from field visits and DAFF monitoring.

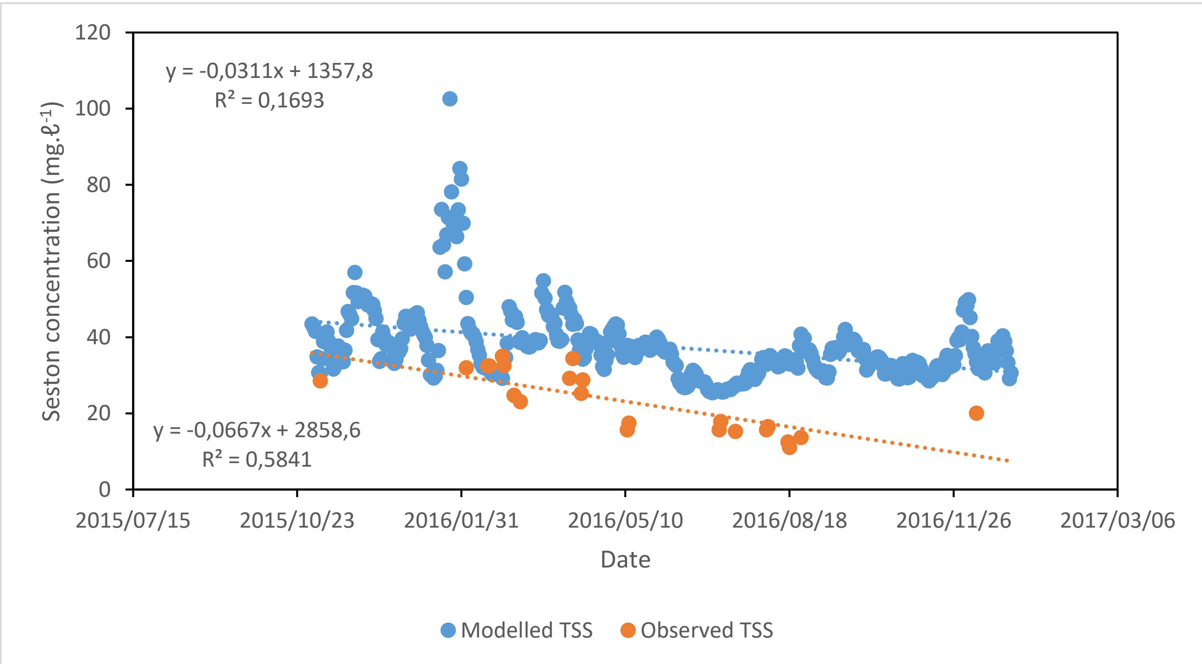

Figure 4.23: Modelled seston concentration (mg.ℓ$^{-1}$) over time from discharge data from Upington weir D7H005, plotted with observed seston concentration (mg.ℓ$^{-1}$) data collected from field visits and DAFF monitoring.

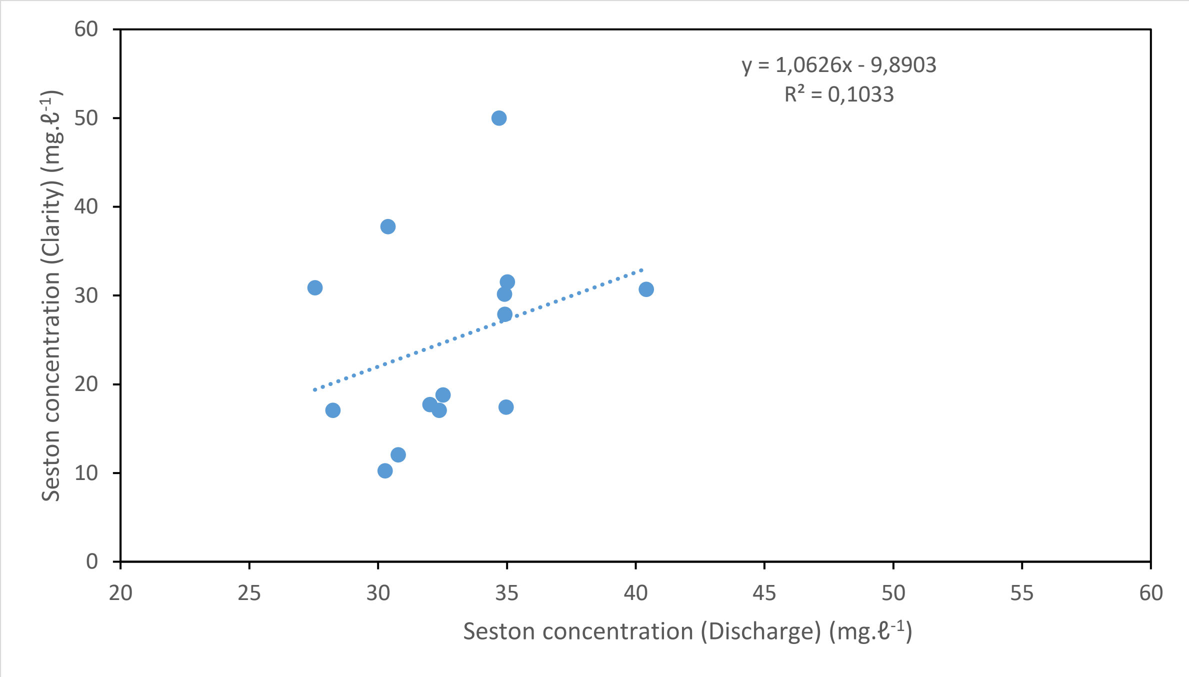

Figure 4.24: Seston concentration (mg.ℓ$^{-1}$) from discharge data obtained from Prieska weir D7H002 versus seston concentrations (mg.ℓ$^{-1}$) from water clarity collected from field visits and DAFF monitoring.

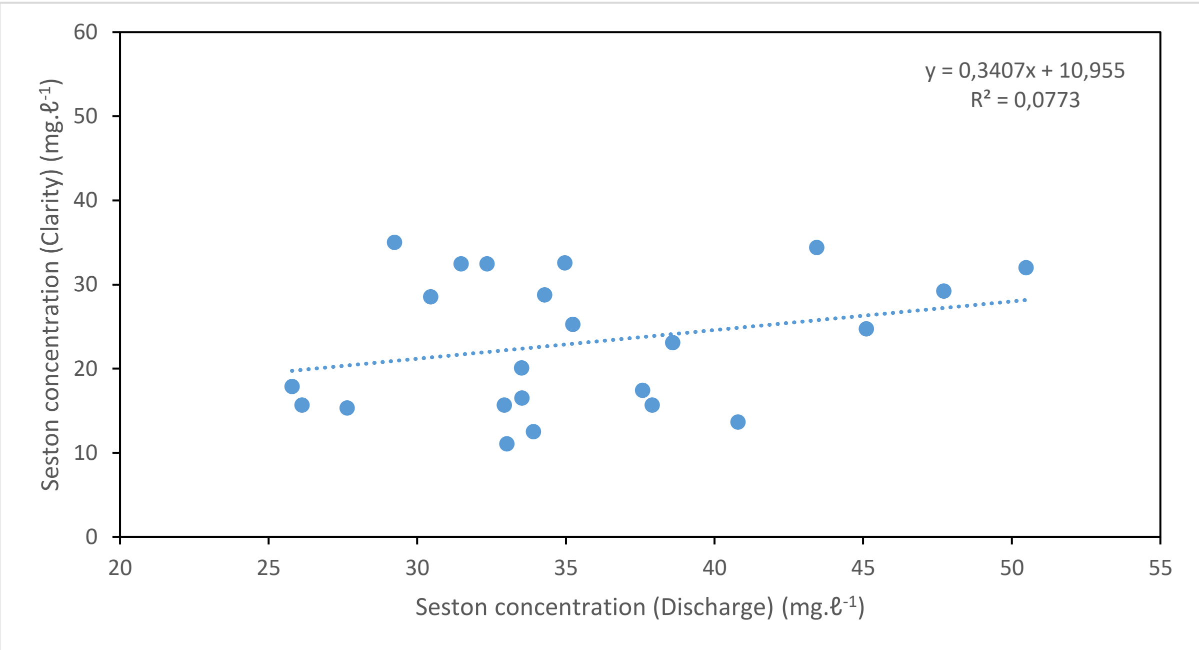

Figure 4.25: Converted seston concentration (mg.ℓ$^{-1}$) from discharge data obtained from Upington weir D7H005 versus converted seston concentrations (mg.ℓ$^{-1}$) from water clarity collected from field visits and DAFF monitoring.

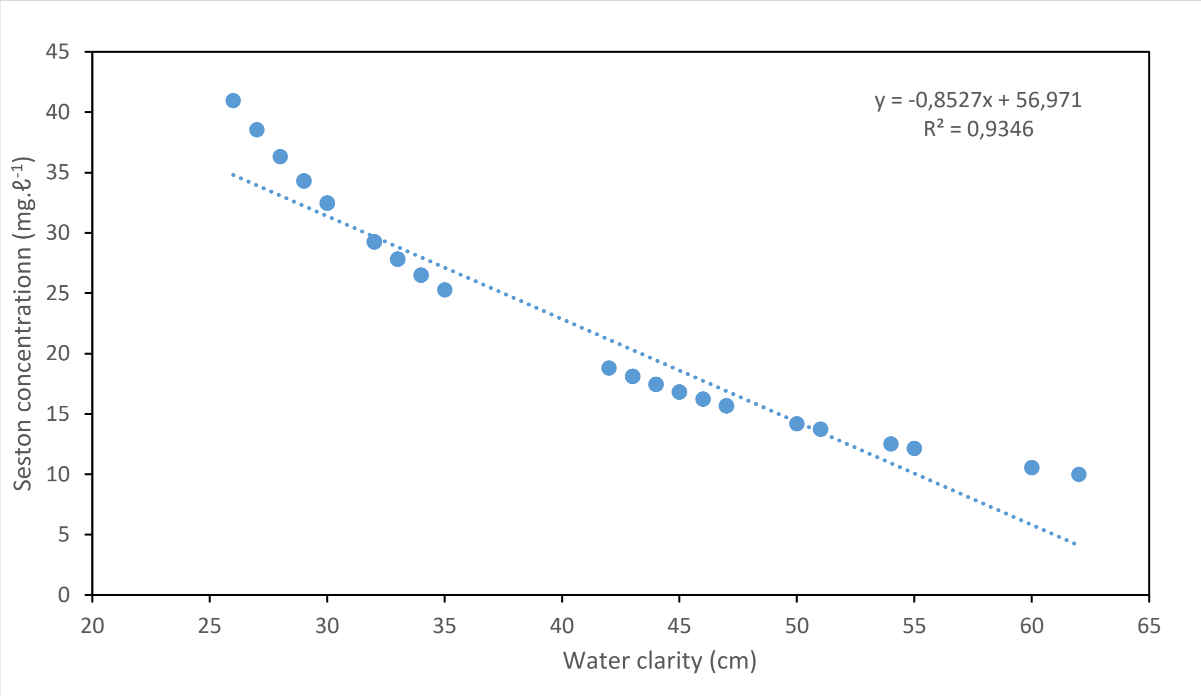

Figure 4.26: Water clarity (cm) versus seston concentration from water clarity (mg.ℓ$^{-1}$) from available DAFF monitoring data.

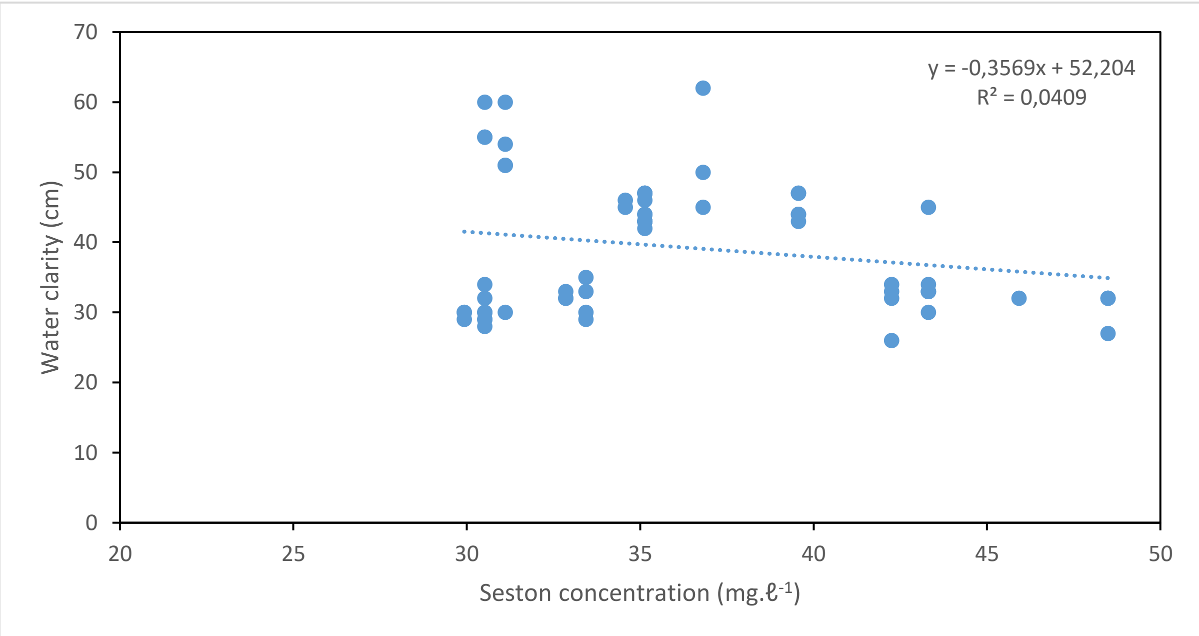

Figure 4.27: Water clarity (cm) versus converted seston concentration from discharge (mg.ℓ$^{-1}$) from available DAFF monitoring data.

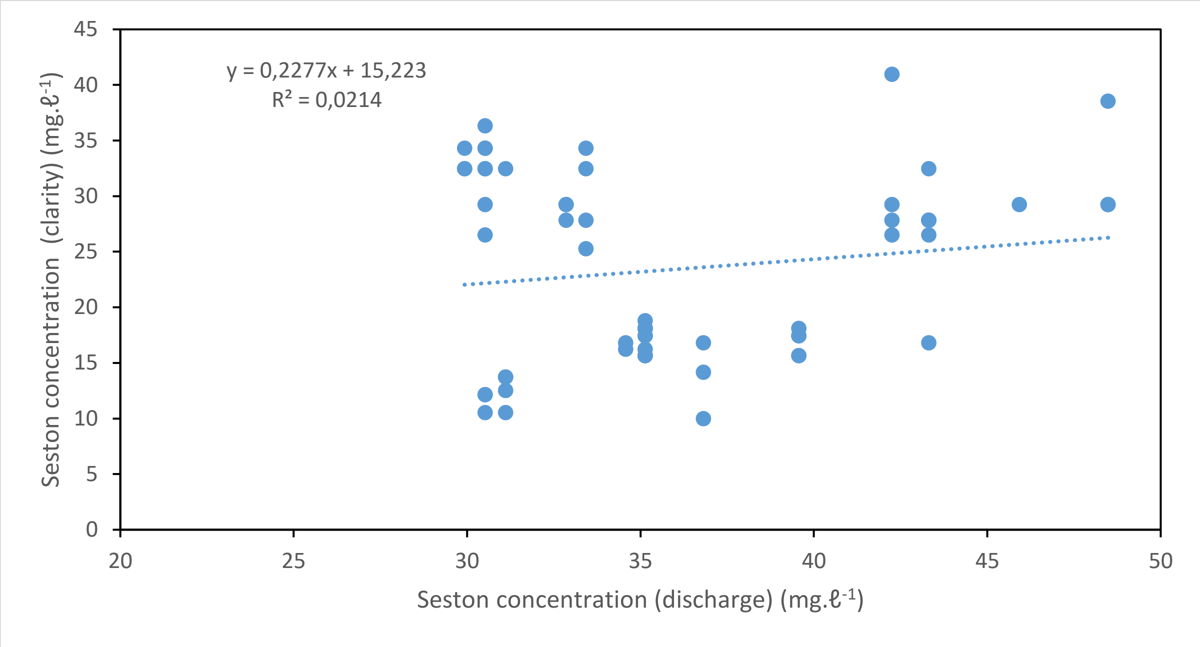

Figure 4.28: Converted seston concentration from water clarity (mg.ℓ$^{-1}$) versus converted seston concentration (mg.ℓ$^{-1}$) from discharge from available DAFF monitoring data.

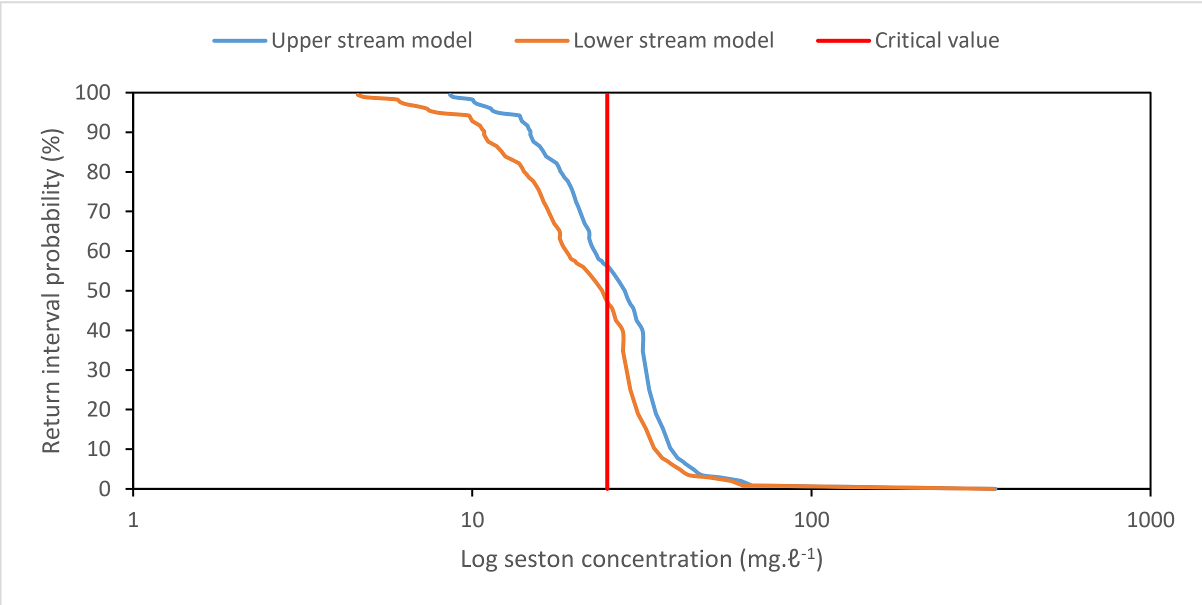

Figure 4.29: Seston concentration return interval calculation from field and DAFF data for node probability calculation in both Bayesian networks (Bns).

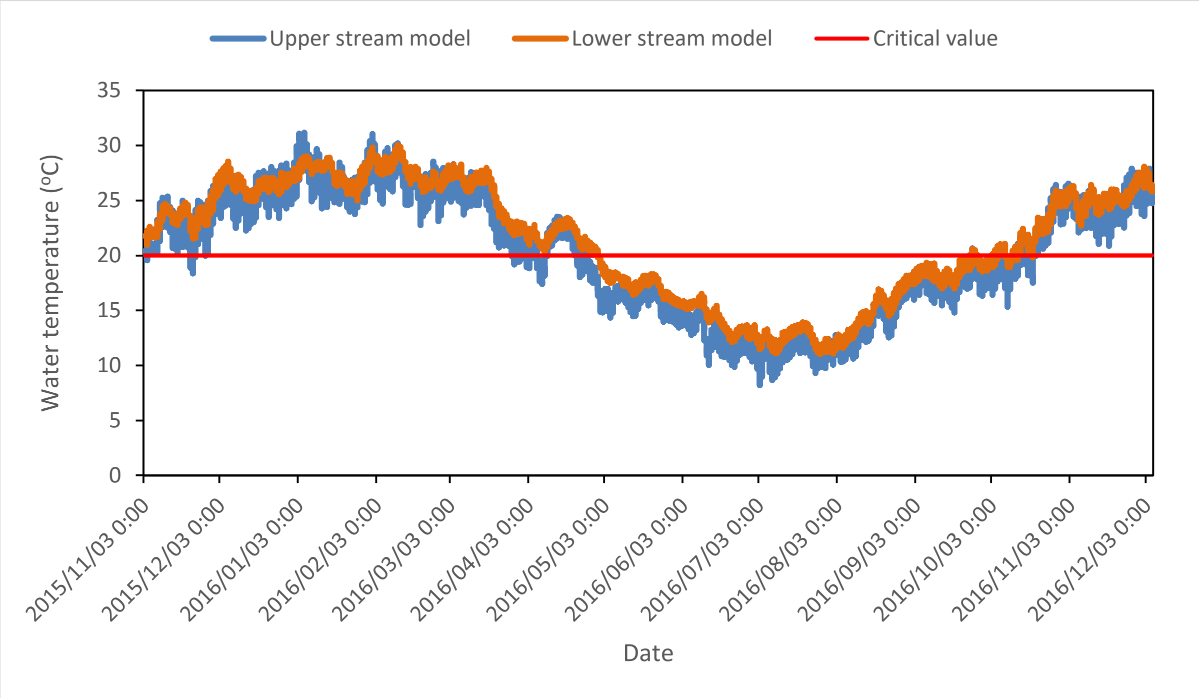

Figure 4.30: Time series water temperature data from HOBO loggers installed during field visits for the upper and lower stream model used to calculate return intervals of values below or exceeding 20° C.

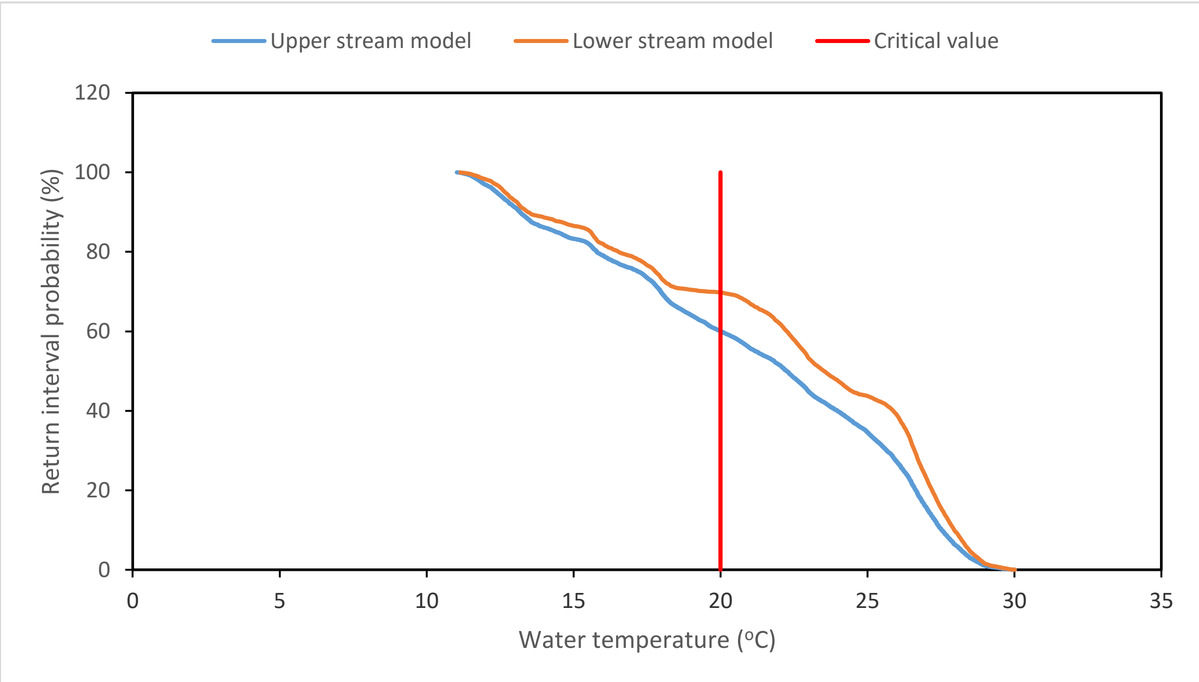

Figure 4.31: Water temperature return interval calculation from HOBO logger data for node probability calculation for both Bayesian network (Bns).

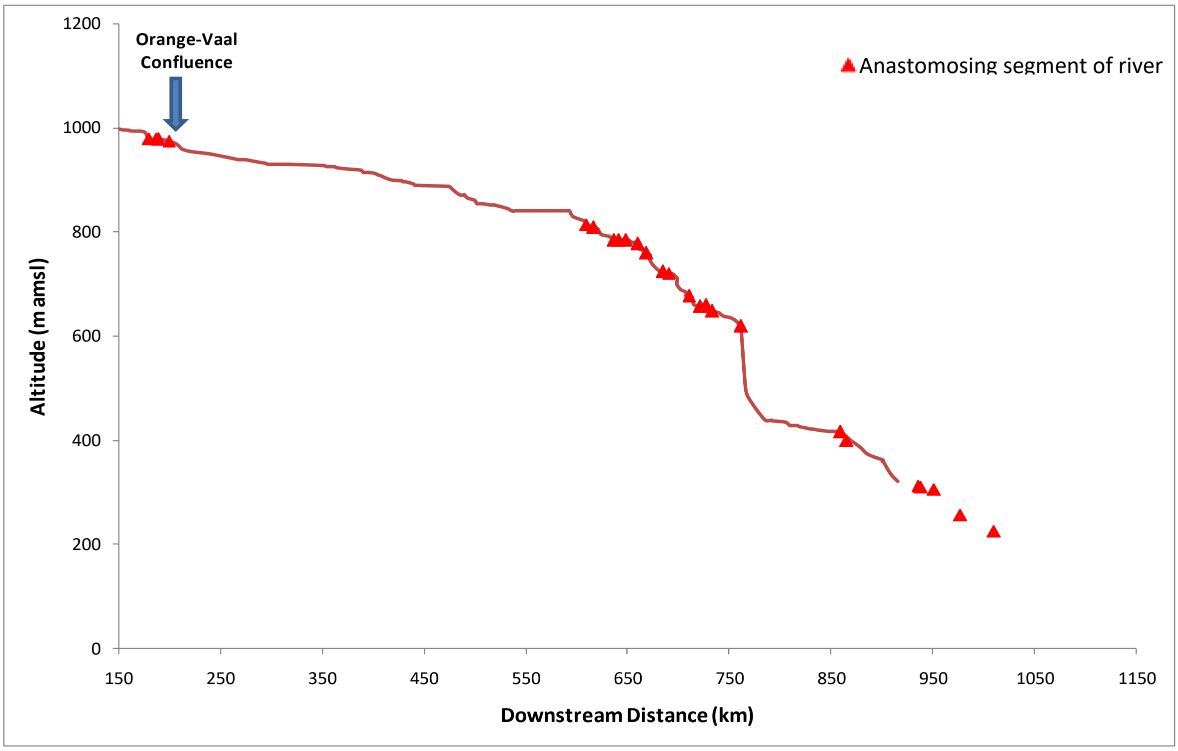

Figure 4.32: Distribution of anastomosing segments on the Orange River, downstream of van der Kloof Dam (Rivers-Moore and Palmer, 2017).

4.5.3. Conditional Probability Tables of the Bayesian networks (Bns)

Data collected from field visits during all sampling periods were collated into case files (Appendix 3 and 4) and were inputted into the Bayesian networks (Bns) to generate Conditional Probability Tables (CPTs) for all 'child' nodes (Table 4.7 - 4.13). The software Netica, used for the construction of the Bns, has learning algorithms which generates the Conditional Probabilities from the case files. For the upper stream model, data collected from sites 1,2 and 9a were combined to create a case file to generate CPTs, and for the lower stream model, data collected from sites 3, 4, 5, 6, 7, 8. 9, 10 and 11 were combined to generate CPTs.

4.5.4. Generation of blackfly outbreak probabilities from the Bayesian networks (Bns)

When the 'parent' node probabilities (Table 4.5 and 4.6) and the Conditional Probability Tables (CPTs) of 'child' nodes (Table 4.7 to 4.13) were combined within the Bayesian networks (Bns), outbreak probabilities were generated for each model. For the upper stream model, there was a 54% probability that there will be a blackfly outbreak occurrence at any given time (Figure 4.33a), whilst for the lower stream model, there was a 47% probability that there will be a blackfly outbreak occurrence at any given time (Figure 4.33b). These were the baseline probabilities that were generated and are not indicative of any specific season or month, but rather a result of all inputted data in the Bns. From these baseline probabilities, monthly outbreak probabilities were generated when observed state of certain nodes were selected based on monthly data.

With the Bns being operational, should a node/s be observed in a certain state, probabilities of other nodes would alter based on this observation, based on linkages and dependencies. For example, when the outbreak probability node is observed to be in a 'low' state, the state 'effective' for the node management changes from a baseline probability of 40.9% (Figure 4.33a) to a probability of 71.6% (Figure 4.34a) for the upper stream model and a baseline probability of 51.1% (Figure 4.33b) to a probability of 94.6% (Figure 4.35a) for the lower stream model. When the outbreak probability node is observed to be in a 'high' state, the state 'poor' for the node management changes from a baseline probability 59.1% (Figure 4.33a) to a probability of 84.8% (Figure 4.34b) for the upper stream model and a baseline probability of 48.9% (Figure 4.33b) to a probability of 97.9% for the lower stream model (Figure 4.35b). During instances of observed outbreaks (outbreak probability in a high state), the most likely species to cause an outbreak would come from the Strong porous group, with a probability of 78.8% (Figure 4.34b) for the upper stream model and 62.4% (Figure 4.35b) for the lower stream model.

Table 4.7a: Conditional probability table (CPT) of the likelihood of algae, which are based on combinations of variables states for seston concentration and water temperature for the upper stream model.

Causative Variable		Algae	
Seston Concentration	**Water Temperature**	**Absent**	**Present**
Low	Cool	50%	50%
Low	Warm	79%	21%
High	Cool	75%	25%
High	Warm	87%	13%

Table 4.7b: Conditional probability table (CPT) of the likelihood of algae, which are based on combinations of variables states for seston concentration and water temperature for the lower stream model.

Causative Variable		Algae	
Seston Concentration	**Water Temperature**	**Absent**	**Present**
Low	Cool	65%	35%
Low	Warm	53%	47%
High	Cool	23%	77%
High	Warm	77%	23%

Table 4.8a: Conditional probability table (CPT) of the likelihood of larvicide efficacy, which are based on combinations of variables states for seston concentration and water temperature for the upper stream model.

Causative Variable		Larvicide efficacy	
Seston Concentration	**Water Temperature**	**Suboptimal**	**Optimal**
Low	Cool	88%	12%
Low	Warm	16%	84%
High	Cool	75%	25%
High	Warm	12%	88%

Table 4.8b: Conditional probability table (CPT) of the likelihood of larvicide efficacy, which are based on combinations of variables states for seston concentration and water temperature for the lower stream model.

Causative Variable		Larvicide efficacy	
Seston Concentration	**Water Temperature**	**Suboptimal**	**Optimal**
Low	Cool	99%	1%
Low	Warm	1%	99%
High	Cool	92%	8%
High	Warm	1%	99%

Table 4.9a: Conditional probability table (CPT) of the likelihood of abiotic conditions, which are based on combinations of variables states for channel type, discharge and seston concentrations for the upper stream model.

Causative Variable			Abiotic	
Channel Type	**Discharge**	**Seston Concentration**	**Unfavourable**	**Favourable**
Anastomosing	Low	Low	75%	25%
Anastomosing	Low	High	50%	50%
Anastomosing	High	Low	50%	50%
Anastomosing	High	High	50%	50%
Single	Low	Low	86%	14%
Single	Low	High	50%	50%
Single	High	Low	33%	67%
Single	High	High	10%	90%

Table 4.9b: Conditional probability table (CPT) of the likelihood of abiotic conditions, which are based on combinations of variables states for channel type, discharge and seston concentrations for the lower stream model.

Causative Variable			Abiotic	
Channel Type	**Discharge**	**Seston Concentration**	**Unfavourable**	**Favourable**
Anastomosing	Low	Low	93%	7%
Anastomosing	Low	High	50%	50%
Anastomosing	High	Low	28%	72%
Anastomosing	High	High	83%	17%
Single	Low	Low	82%	18%
Single	Low	High	96%	4%
Single	High	Low	29%	71%
Single	High	High	24%	76%

Table 4.10a: Conditional probability table (CPT) of the likelihood of biotic conditions, which are based on combinations of variables states for algae and reeds for the upper stream model.

Causative Variable		Biotic	
Algae	**Reeds**	**Weak**	**Strong**
Absent	Absent	75%	25%
Absent	Present	4%	96%
Present	Absent	50%	50%
Present	Present	88%	12%

Table 4.10b: Conditional probability table (CPT) of the likelihood of biotic conditions, which are based on combinations of variables states for algae and reeds for the lower stream model.

Causative Variable		Biotic	
Algae	**Reeds**	**Weak**	**Strong**
Absent	Absent	50%	50%
Absent	Present	100%	0%
Present	Absent	98%	2%
Present	Present	99%	1%

Table 4.11a: Conditional probability table (CPT) of the likelihood of the *Simulium* Group Complex, which are based on combinations of variables states for abiotic and biotic conditions for the upper stream model.

Causative Variable		***Simulium* Group Complex**	
Abiotic	**Biotic**	**Standard**	**Strong Porous**
Unfavourable	Weak	40%	60%
Unfavourable	Strong	50%	50%
Favourable	Weak	50%	50%
Favourable	Strong	5%	95%

Table 4.11b: Conditional probability table (CPT) of the likelihood of the *Simulium* Group Complex, which are based on combinations of variables states for abiotic and biotic conditions for the lower stream model.

Causative Variable		***Simulium* Group Complex**	
Abiotic	**Biotic**	**Standard**	**Strong Porous**
Unfavourable	Weak	66%	34%
Unfavourable	Strong	46%	54%
Favourable	Weak	43%	57%
Favourable	Strong	9%	91%

Table 4.12a: Conditional probability table (CPT) of the likelihood of poor or effective management, which are based on combinations of variables states for larvicide efficacy and spraying for the upper stream model.

Causative Variable		Management	
Larvicide efficacy	**Spraying**	**Poor**	**Effective**
Suboptimal	Unsuccessful	80%	20%
Suboptimal	Successful	11%	89%
Optimal	Unsuccessful	94%	6%
Optimal	Successful	14%	86%

Table 4.12b: Conditional probability table (CPT) of the likelihood of poor or effective management, which are based on combinations of variables states for larvicide efficacy and spraying for the lower stream model.

Causative Variable		Management	
Larvicide efficacy	**Spraying**	**Poor**	**Effective**
Suboptimal	Unsuccessful	98%	2%
Suboptimal	Successful	1%	99%
Optimal	Unsuccessful	99%	1%
Optimal	Successful	6%	94%

Table 4.13a: Conditional probability table (CPT) of the likelihood of a blackfly outbreak, which are based on combinations of variables states for *Simulium* Group Complex, management, and water temperature for the upper stream model.

Causative Variable			Outbreak Probability	
***Simulium* Group Complex**	**Management**	**Water temperature**	**Low**	**High**
Standard	Poor	Cool	50%	50%
Standard	Poor	Warm	50%	50%
Standard	Effective	Cool	7%	33%
Standard	Effective	Warm	83%	17%
Strong Porous	Poor	Cool	20%	80%
Strong Porous	Poor	Warm	6%	94%
Strong Porous	Effective	Cool	83%	17%
Strong Porous	Effective	Warm	80%	20%

Table 4.13b: Conditional probability table (CPT) of the likelihood of a blackfly outbreak, which are based on combinations of variables states for *Simulium* Group Complex, management, and water temperature for the lower stream model.

Causative Variable			Outbreak Probability	
***Simulium* Group Complex**	**Management**	**Water temperature**	**Low**	**High**
Standard	Poor	Cool	28%	72%
Standard	Poor	Warm	6%	94%
Standard	Effective	Cool	98%	2%
Standard	Effective	Warm	99%	1%
Strong Porous	Poor	Cool	2%	98%
Strong Porous	Poor	Warm	1%	99%
Strong Porous	Effective	Cool	96%	4%
Strong Porous	Effective	Warm	99%	1%

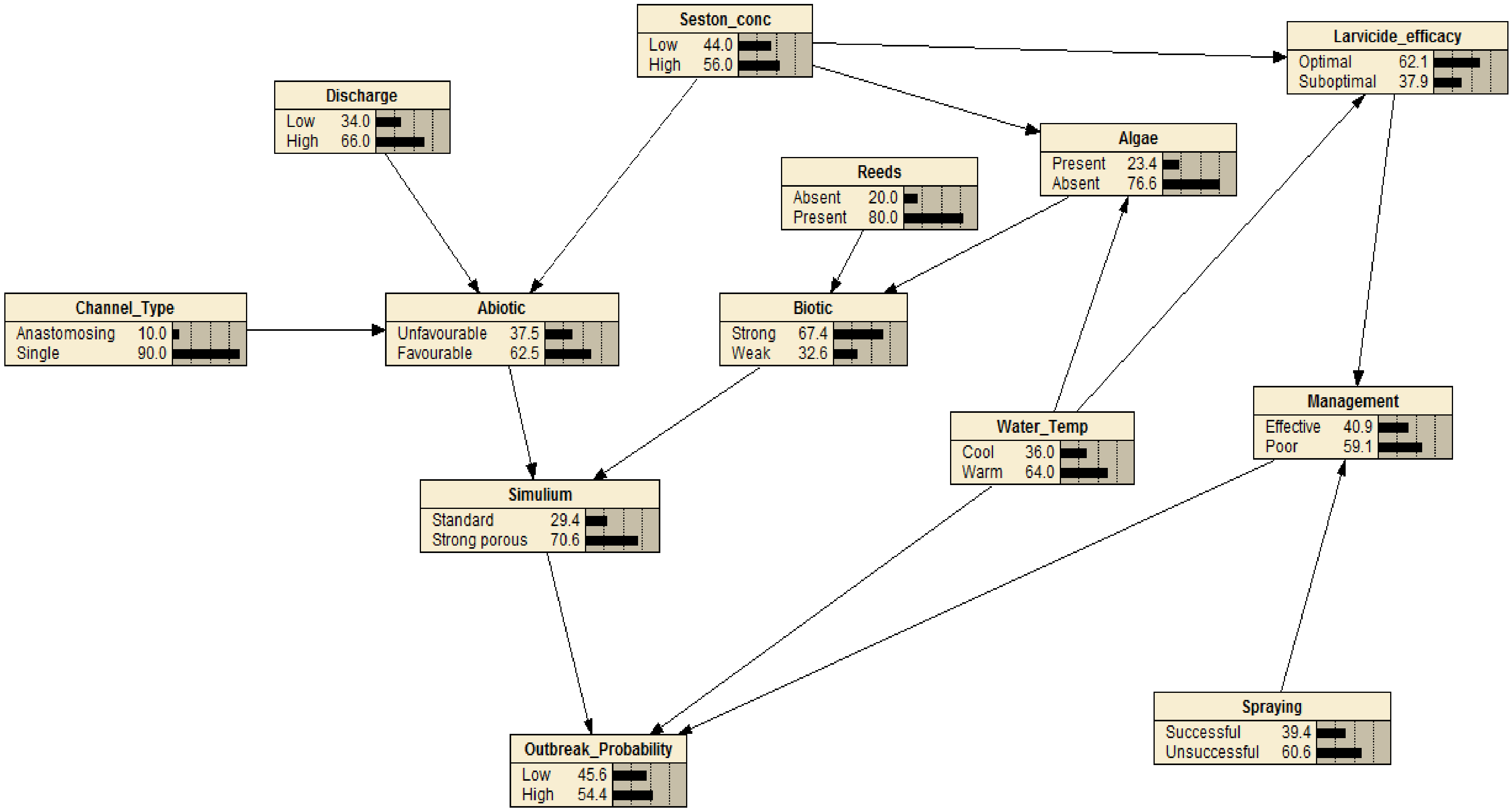

Figure 4.33a: Bayesian network (Bn) diagram for the upper stream model, which shows the baseline blackfly outbreak probability and the relative conditional likelihoods of the driver variables in the Orange River system.

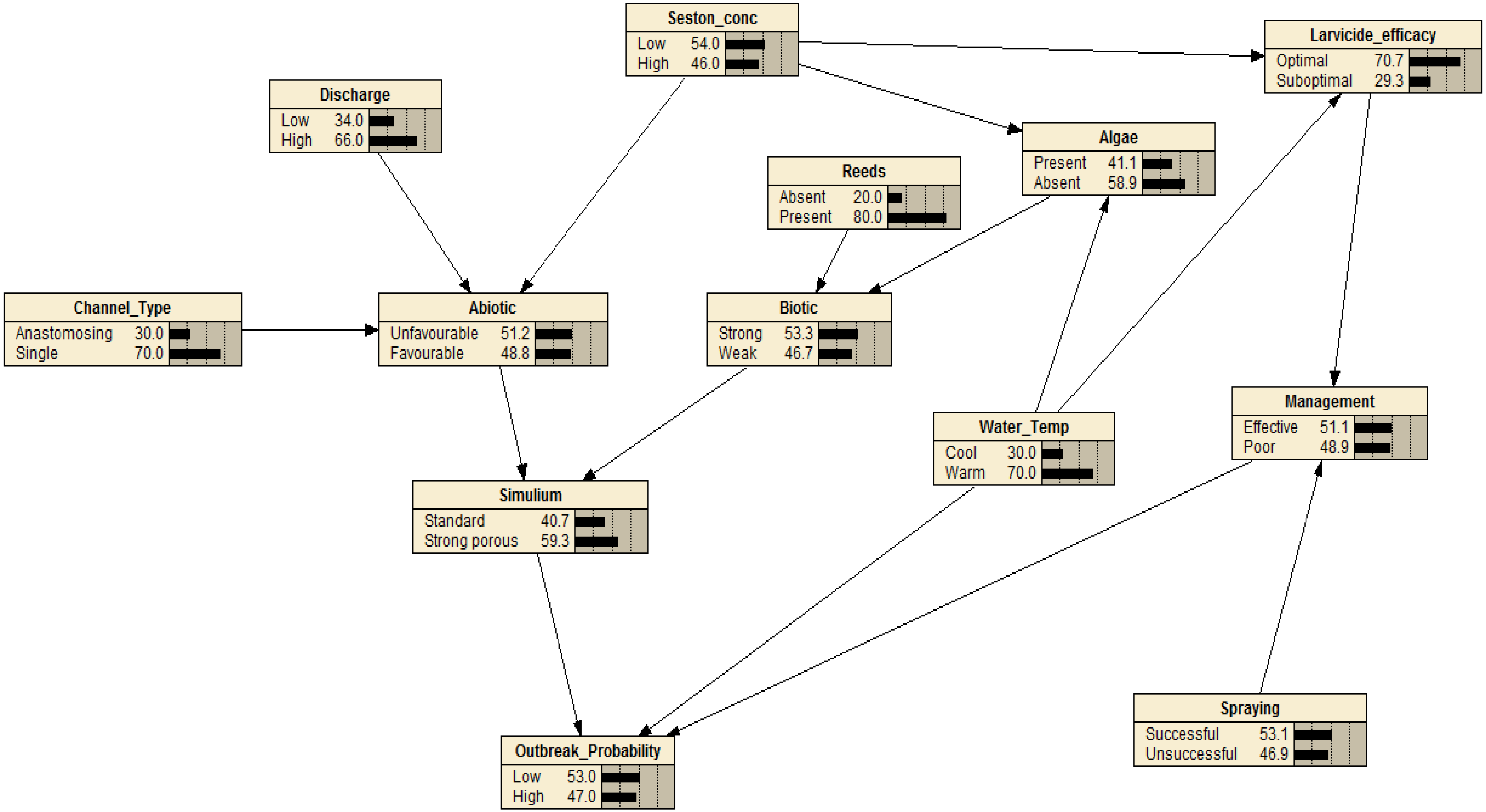

Figure 4.33b: Bayesian network (Bn) diagram for the lower stream model, which shows blackfly outbreak probability and the relative conditional likelihoods of the driver variables in the Orange River system.

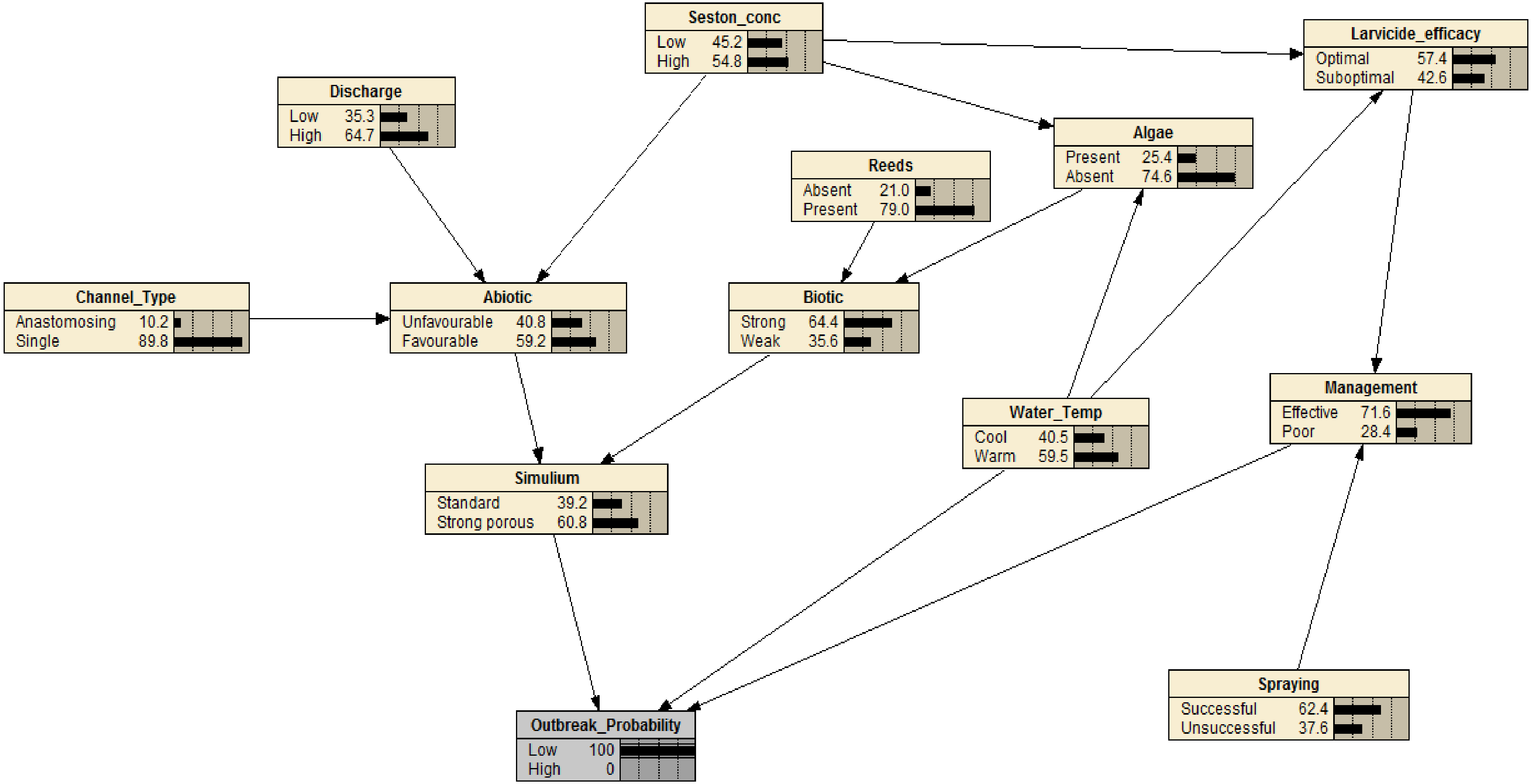

Figure 4.34a: Bayesian network (Bn) diagram for the upper stream model, which shows blackfly outbreak probability (for which the observed effect is confirmed to be an absence of an outbreak) and the relative conditional likelihoods of the driver variables in the Orange River system.

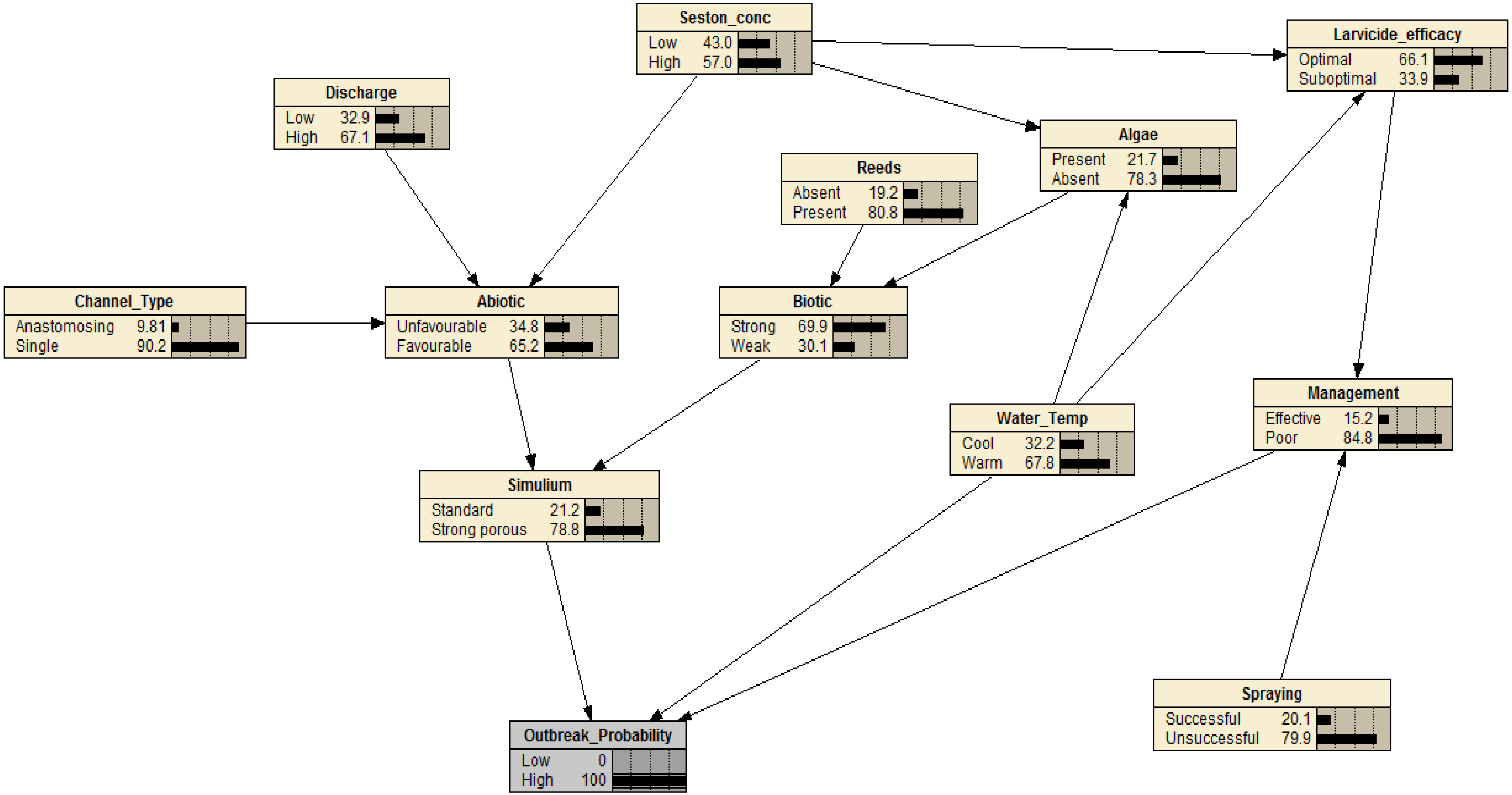

Figure 4.34b: Bayesian network (Bn) diagram for the upper stream model, which shows blackfly outbreak probability (for which the observed effect is a confirmed outbreak) and the relative conditional likelihoods of the driver variables in the Orange River system.

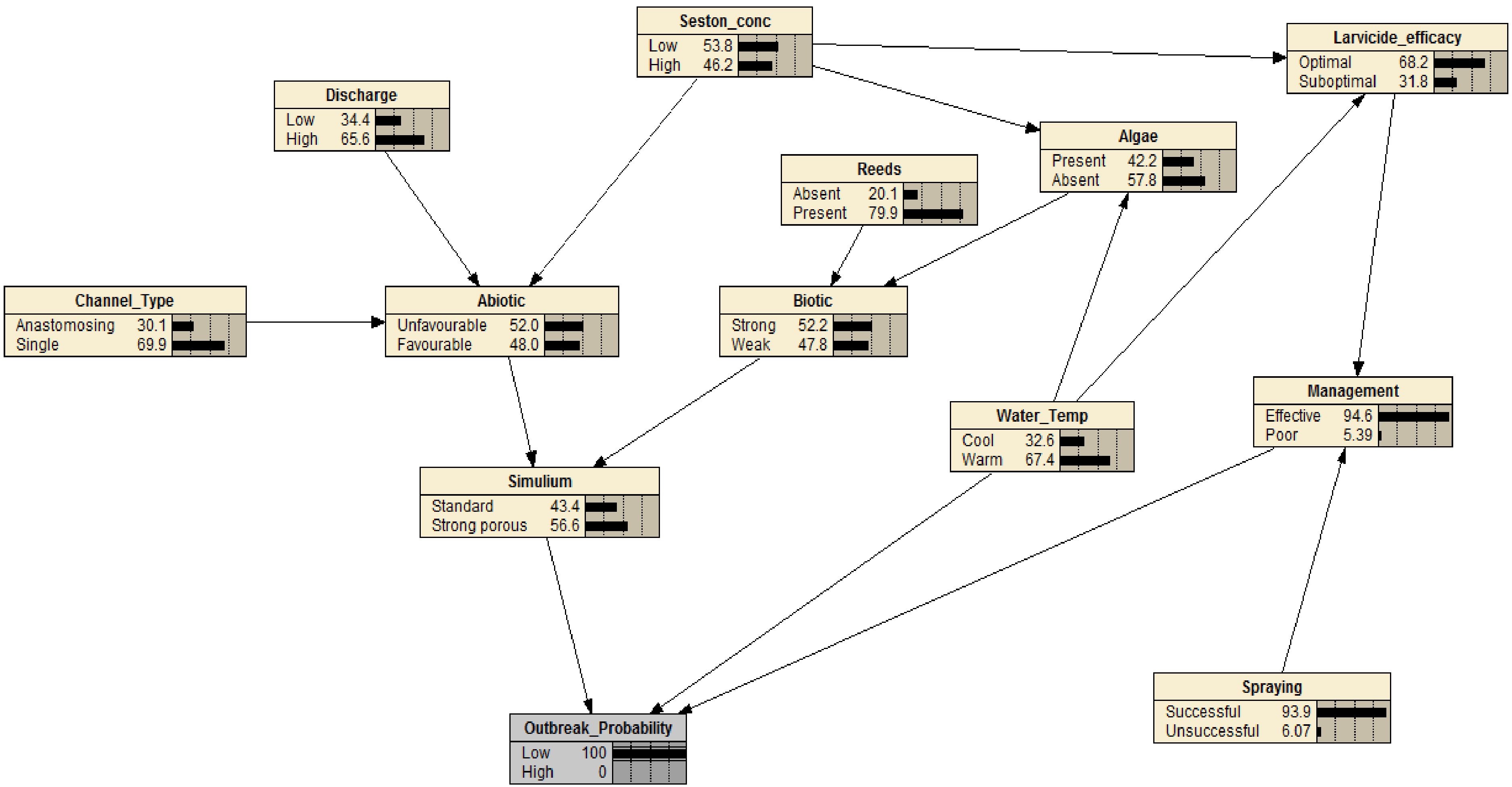

Figure 4.35a: Bayesian network (Bn) diagram for the lower stream model, which shows blackfly outbreak probability (for which the observed effect is confirmed to be an absence of an outbreak) and the relative conditional likelihoods of the driver variables in the Orange River system.

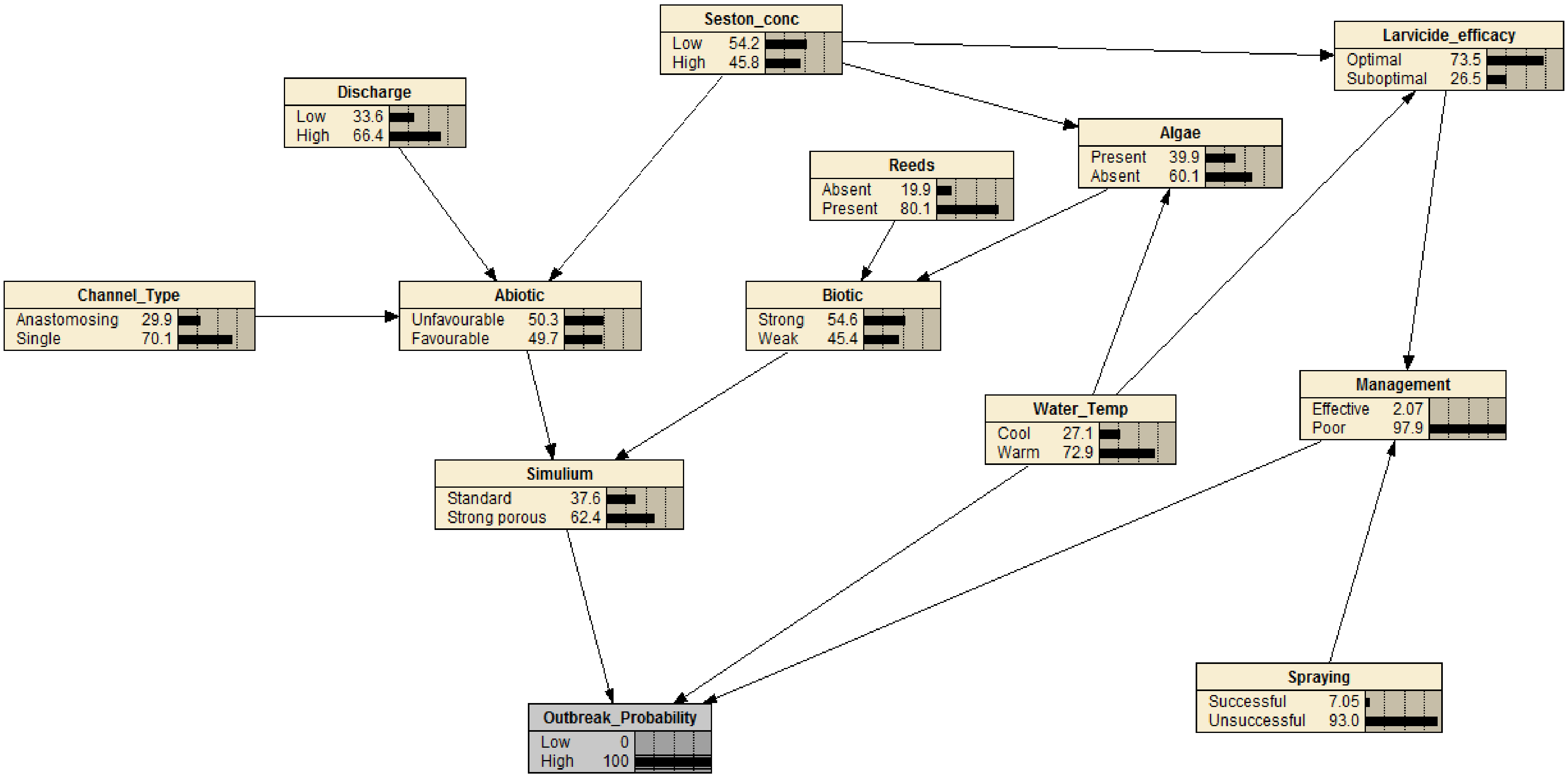

Figure 4.35b: Bayesian network (Bn) diagram for the lower stream model, which shows blackfly outbreak probability (for which the observed effect is a confirmed outbreak) and the relative conditional likelihoods of the driver variables in the Orange River system.

4.5.5. Temporal blackfly outbreak probabilities

Temporally, each model was run monthly (twelve times), and produced blackfly outbreak for each month of the year. For both models, observed states of the nodes ‘discharge’, ‘seston concentration and ‘water temperature’ were used to generate monthly outbreak probabilities. Observations of other node states were not used as there was no available monthly data to base observations on. Monthly discharge (Figure 4.19 and 4.20) and water temperature data (Figure 4.30) were used to identify observed states for ‘discharge’ and ‘water temperature’ nodes, whilst observations on ‘seston concentration’ was based on Palmer (1997) seasonal variation in seston concentration (Table 4.14). Blackfly outbreak probabilities were higher for the upper stream model, with the highest probabilities expected in the months of January, February, March, April, November and December for both models (Figure 4.36). The outbreak probabilities during these month were 59.9% for the upper stream model and 49.2% for the lower stream model (Figure 4.36). The lowest outbreak probabilities were expected during the months of June to September (Figure 4.36). The probabilities during these months were 49.2% for the upper stream model and 42.2% for the lower stream model. Monthly outbreaks generated between the two Bns were significantly different ($p=0.0003$, $P < 0.05$). Outbreak probabilities for both models were indicative of seasonality, therefore, the temporal resolution of the Bns of monthly outbreak probabilities were reduced to seasonal outbreak probabilities (Figure 4.37). Statistically, seasonal blackfly outbreak probabilities generated for the upper and lower stream models were significantly different ($p = 0.003$, $P < 0.05$).

Seasonal outbreak probabilities were generated under the observations of successful and unsuccessful spraying of larvicides for both models (Figure 4.38 and 4.39). Outbreak probabilities are higher when the spraying node is observed to be unsuccessful for both models (Figure 4.38 and 4.39). Should spraying be unsuccessful, outbreak probabilities in summer were 80.3% and 96.8%, whilst winter had outbreak probabilities of 59.4% and 83.9% for the upper and lower stream models respectively (Figure 4.38 and 4.39). Should spraying be successful, outbreak probabilities in summer were 28.5% and 7.06%, whilst winter had outbreak probabilities of 28.7% and 4.22% for the upper and lower stream models respectively (Figure 4.38 and 4.39). Therefore, the Bns are highly indicative that blackfly outbreaks are reliant on whether spraying is successful or unsuccessful.

Table 4.14: Observed monthly states of seston concetration, discharge and water temperature, used for both models. Monthly states were derived from relevant literature (seston concentration), weirs (discharge) and collected data from the field (water temperature).

Month	Seston concentration	Discharge	Water Temperature
January	High	High	High
February	High	High	High
March	High	High	High
April	High	High	High
May	High	High	Low
June	Low	Low	Low
July	Low	Low	Low
August	Low	Low	Low
September	Low	Low	Low
October	Low	Low	High
November	High	High	High
December	High	High	High

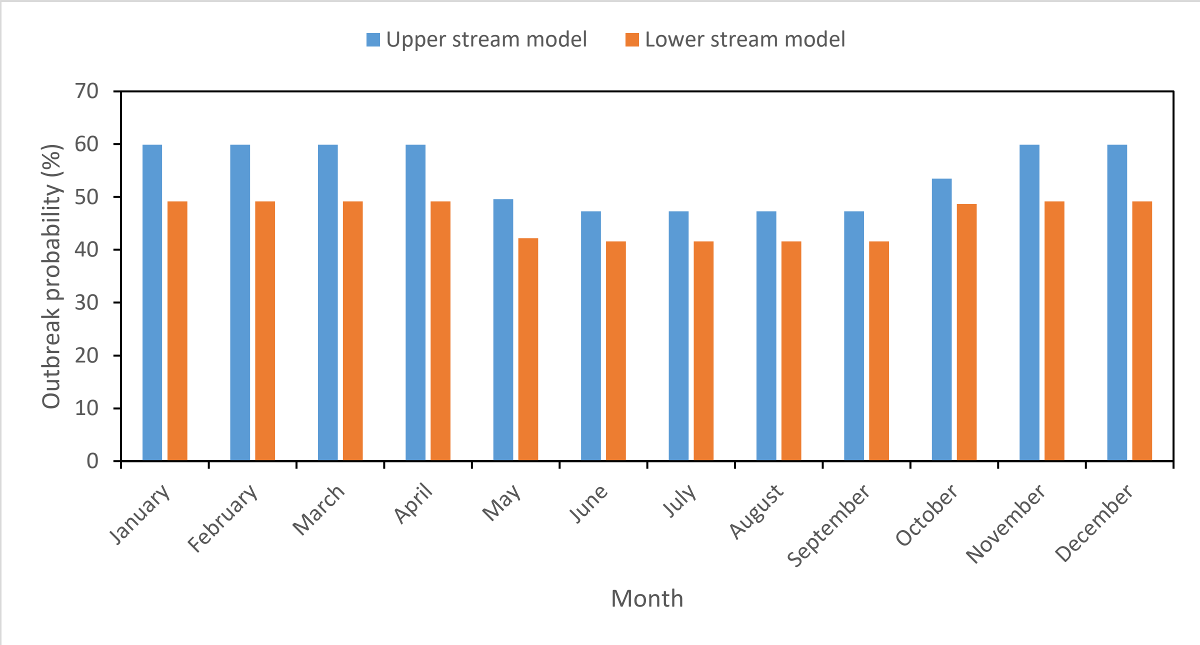

Figure 4.36: Monthly blackfly outbreak probabilities (%) for both models, based on observed monthly states for the nodes discharge, seston concentration and water temperature within the Bayesian networks (Bns).

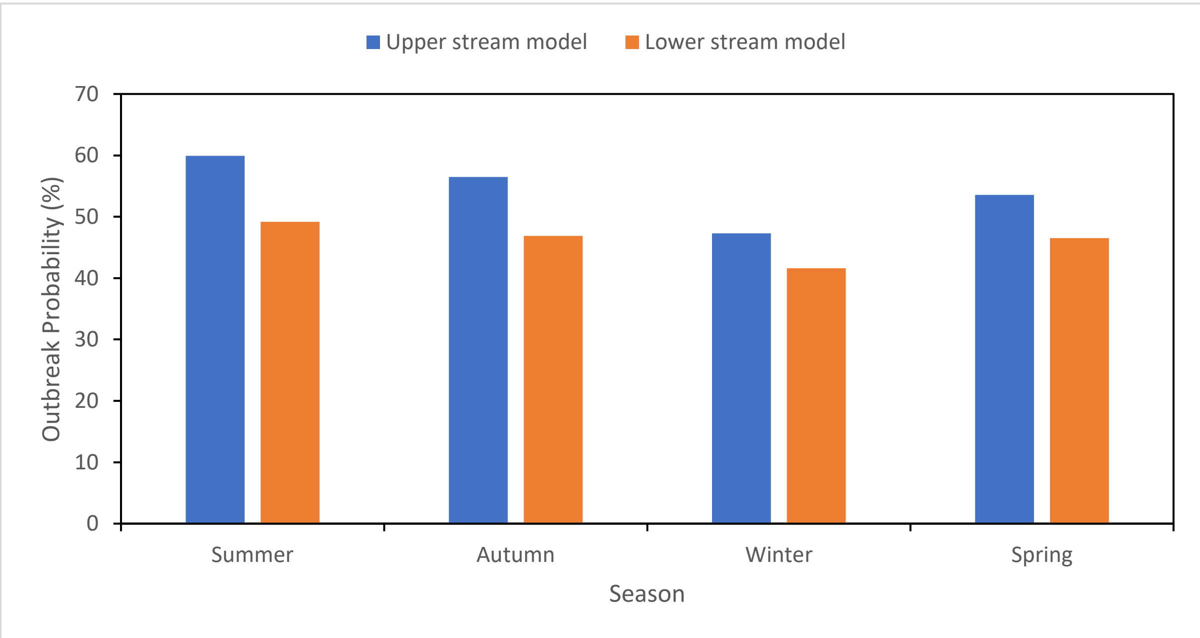

Figure 4.37: Seasonal blackfly outbreak probabilities (%) for both models, based on observed monthly states for the nodes discharge, seston concentration and water temperature within the Bayesian networks (Bns).

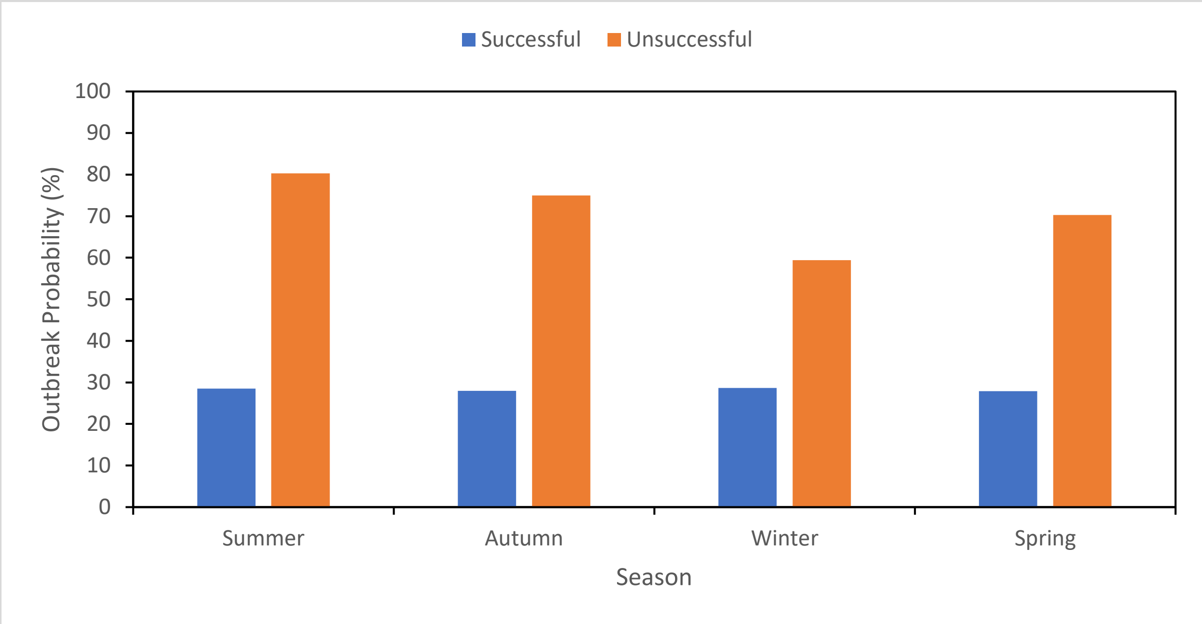

Figure 4.38: Seasonal blackfly outbreak probabilities (%) depending on whether the observed state for the node 'spraying' was successful or unsuccessful for the upper stream model, based on observed monthly states for the nodes discharge, seston concentration and water temperature within the Bayesian networks (Bns).

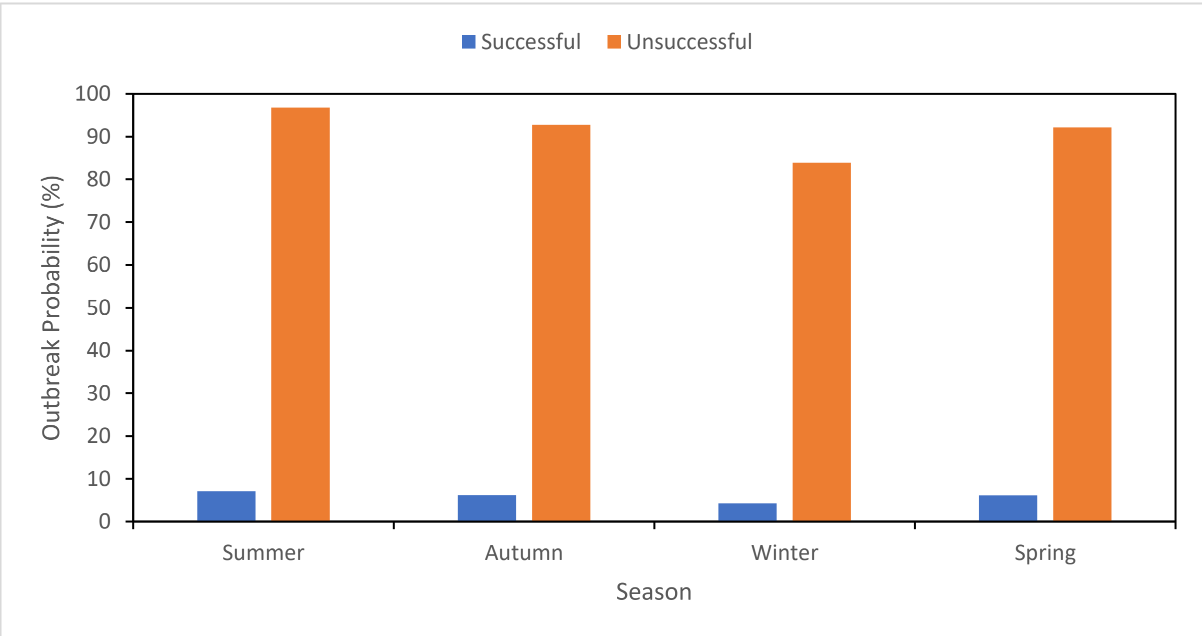

Figure 4.39: Seasonal blackfly outbreak probabilities (%) depending on whether the observed state for the node 'spraying' was successful or unsuccessful for the lower stream model, based on observed monthly states for the nodes discharge, seston concentration and water temperature within the Bayesian networks (Bns).

4.5.6. Model verification

Blackfly outbreak probabilities generated from the upper and lower stream Bayesian networks (Bns) (Figure 4.36), indicate that outbreak probabilities are seasonal based. Highest outbreak probabilities are expected during summer, whilst the lowest probabilities are expected during winter (Figure 4.37). However, when comparing probabilities generated from the Bns to DAFF blackfly monitoring data and data collected during sampling periods, which were collected using density scores based on Palmer's (1994) logarithmic 10-point scale of blackfly abundance between 2009 and 2016 (Figure 4.40), a contrast was apparent. Density scores collected between 2009-2016 show trends that the highest densities of blackfly larvae and pupae are in the months of May to September, whilst the lowest were during the summer months (Figure 4.40). From the density data, the highest monthly outbreak probabilities were 87.5% in September 2011, 78% in May 2013 and 82% in August 2014 (Figure 4.40). For the warmer months (November to March), the outbreak probabilities ranged from 53% to 20% throughout all the years which indicates a lower outbreak probability for these months based on the density data (Figure 4.40). A possible reason for this is due to the lag time in the blackfly life cycle from larvae phase to the adult phase, which varies depending on abiotic conditions. Therefore, although larvae density is highest in winter, the actual adult emergence will occur at a later stage.

However, when the density data from 2009-2016 were combined, although it was still apparent that outbreak probabilities were highest during winter compared to both Bns highest being predicted in summer (Figure 4.41), outbreak probabilities showed no significant differences between density outbreak probabilities and Bns generated probabilities. Statistically, the upper stream model showed no significant difference to the density data ($p = 0.23$, $P > 0.05$), and this was the same for the lower stream model as it showed no significant difference to the density data ($p = 0.80$, $P > 0.05$). Therefore, due to there being no significant differences between outbreak probabilities generated from the Bns and density data, it can be assumed that the Bns are able to produce accurate outbreak probabilities.

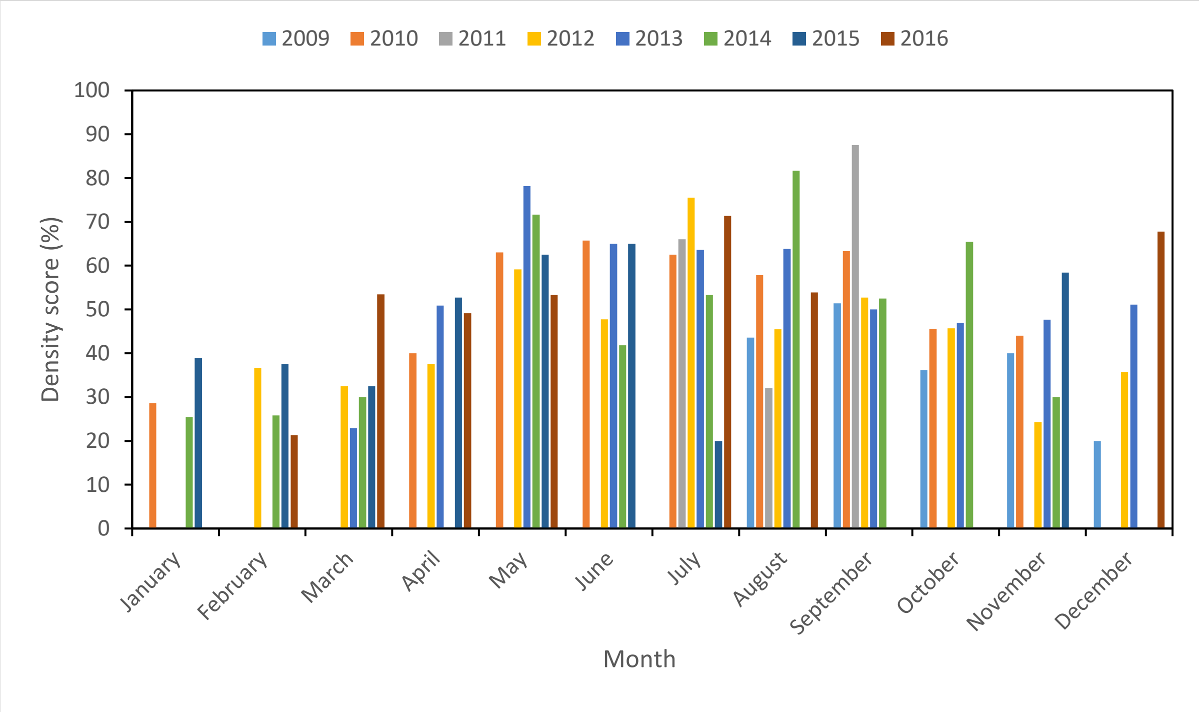

Figure 4.40: Monthly density scores (%) from Palmer's (1994) 10-point scale of blackfly abundance obtained and collated from data collected during sampling periods and 14 Department of Fishery and Forestry's (DAFF) blackfly monitoring sites along the middle to lower sections of the Orange River.

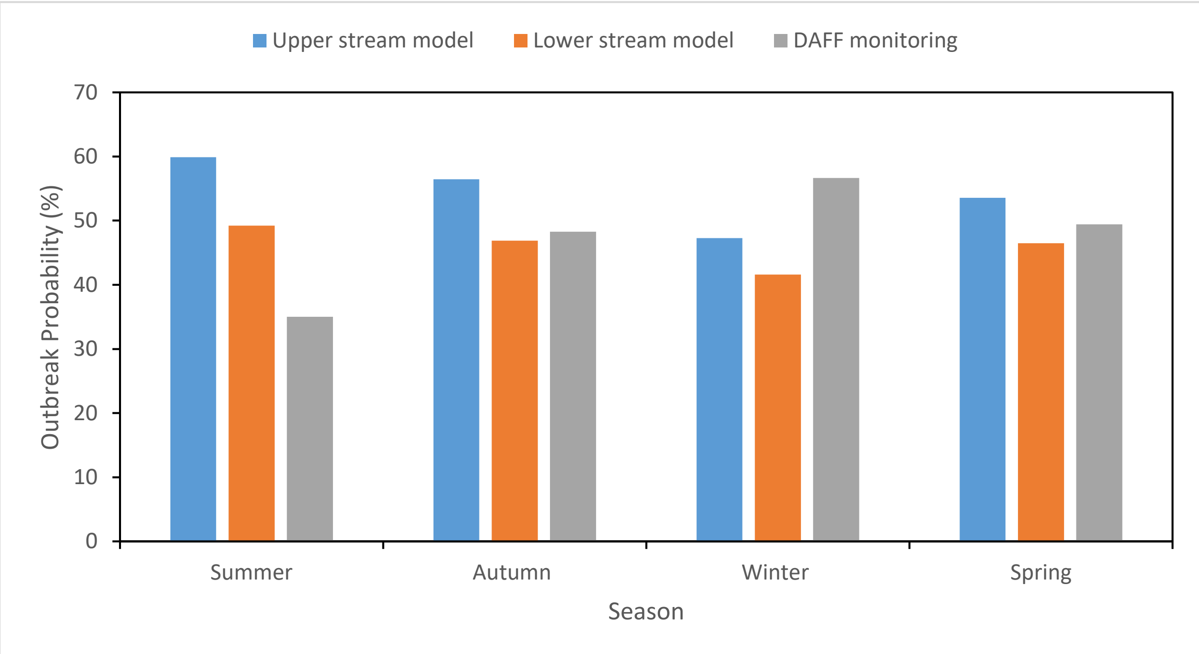

Figure 4.41: Comparison of seasonal blackfly outbreak probabilities generated for each spatial Bayesian network (Bn) to DAFF combined monitoring data from 2009-2016.

4.5.7. Scenario analysis

With potential changes in environmental variables and conditions due to climate change, predicting blackfly outbreak probabilities based on these changes in discharge and water temperature are important for management. With the baseline blackfly outbreak probabilities for the upper and lower stream probabilities being 54.4% and 47% respectively, blackfly outbreak probabilities were generated within the Bayesian networks (Bn) for the following scenarios: an increase in discharge, an increase in water temperature and an increase in discharge and water temperature (Figure 4.42). The upper stream model had higher predicted outbreak probabilities than the lower stream model (Figure 4.42). Scenarios of increased discharge and water temperatures had the highest outbreak probabilities of all the scenarios with increase in discharge having the lowest for both models (Figure 4.42).

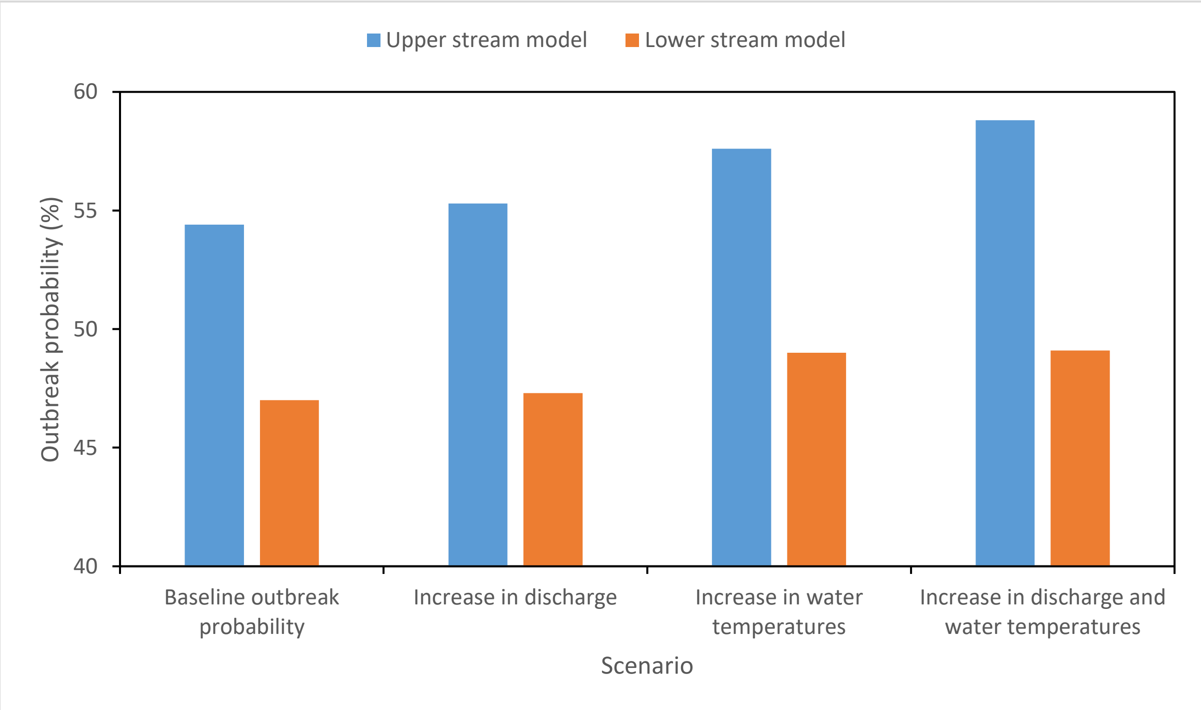

Figure 4.42: Blackfly outbreak probabilities generated from the Bayesian networks (Bns) of both models for scenarios of increased discharge, increased water temperature and a combination of both.

4.6. Model limitations

While the Bayesian networks (Bns) generated valid outbreak probabilities for the lower to middle reaches of the Orange River, there were limitations during the construction phase. Long-term environmental data at each site were not available. Collection of a long-term dataset at each site of; flow velocity, discharge and seston concentration may have been able to identify further splits in ordination which may have resulted in more spatial models. Additionally, long-term data was needed to help improve accuracy for state probabilities for nodes, as seston concentration state probabilities had limited data from which return intervals were calculated. There were no quantitative data for the nodes; algae, reeds, larvicide efficacy, management and spraying. This lead to assumed values being used in case files that were used to populate the Conditional Probability Tables (CPTs).

Quantitative data would have been more desirable than assumed values used within the CPTs and state probabilities. When verifying the probabilities generated from the models, blackfly adult emergence data were not available and therefore larvae and pupae density data were used for model verification.

4.7. Conclusion

Abiotic and biotic data collected at all sites aided in the construction of the Bayesian networks (Bns). Seasonal variations are apparent for seston concentration ($mg.\ell^{-1}$) and conductivity ($\mu S.cm^{-1}$). High abundance of blackfly was found, with the July sampling season containing the highest number of blackfly collected. Most common species were *S.chutteri, S.damnosum* and *S.adersi*. Palmer and Craig's (2000) model illustrates that species can be grouped based on labral fan structure which is based on adaptations and preferences for flow velocity ($m.s^{-1}$) and seston concentration ($mg.\ell^{-1}$). *S.chutteri* and *S.damnosum* belong to the group 'strong porous', whilst *S.adersi* belongs to the group 'standard'. These two groups were used for the '*Simulium*' node within the Bns. Critical values or thresholds were obtained for the 'parent' nodes; discharge, seston concentration and water temperature. Return intervals were calculated based off these values, to populate states probabilities within the Bns. Spatially, sites were grouped based on water temperature data and showed two distinct groups and were classed as the 'upper stream model' and the 'lower stream model'. Seasonal outbreak probabilities generated for both models suggest a significantly higher outbreak probability for the upper stream model than the lower stream model. Scenario analysis based on observed states indicate that the blackfly outbreak probabilities will increase under the following conditions: An increase in discharge, water temperature and a combination of both.

Chapter Five

Discussion

5.1. Introduction

The aim of the research was to refine predicted blackfly outbreak probabilities for selected sites along the lower to middle reaches of the Orange River using Bayesian networks (Bns). This was achieved when qualitative components were constructed through identifying key nodes and linkages between nodes, and the quantitative components were constructed through collected abiotic and biotic data which were key parameters for the Bns. Rivers-Moore *et al*., (2014) preliminary Bn was improved upon with additional nodes incorporated into the models of known variables which affect blackfly outbreaks. These variables were; water temperature, algae, reeds and larvicide efficacy.

Rivers-Moore *et al* (2014) had one standard Bn which was applied to all sampling sites, whereas for our research abiotic data collected at each site were used to determine the spatial resolutions of the Bns for whether more than one Bn was needed or not. Biotic data were collected and determined that the *Simulium* group complex most likely to cause an outbreak were from the strong porous or standard complex group. The *Simulium* node was changed from being either *S.chutteri* or *S.impukane* (Rivers-Moore *et al*., 2014), to strong porous or standard complex group.

Calculations of state probabilities and Conditional Probability Tables (CPTs) of nodes were done with available quantitative data and assumed values from literature when necessary. Case files were constructed from data collected during sampling periods, and this was an improvement from the population methods used by Rivers-Moore *et al*., (2014) who used expert opinion data to populate CPTs, which may be subjective and less likely to be accepted than population by quantitative case files. Case files were created from the abiotic and biotic data and Conditional Probabilities Tables (CPTs) of 'child' nodes were populated which resulted in a functioning model which could predict blackfly outbreak probabilities for each Bn under current conditions and for future scenarios where change occurs.

5.2. Abiotic variations

Seston concentration (mg.ℓ^{-1}) did not show distinct variations between sites, but did exhibit seasonal variations with sampling periods in the warmer months experiencing higher seston concentrations in comparison to the colder months (Figure 4.1). These trends were mentioned by Palmer (1997). Berg and Newell (1986) are supportive of this as suggestions were made that winter had much lower seston concentrations than summer months. Berg and Newell (1986) link seasonal variations to precipitation, and with summer rainfall, higher seston concentrations are expected. With higher rainfall, flow velocities increase which allow for motion of particles in the stream to occur and leads to greater suspended loads in the water (Webster *et al*., 1987). When there are low rainfalls, lower velocities results in less motion of particles in the stream which reduces the suspended load and increases the bed load (Webster *et al*., 1987). Therefore, flow velocity and seston concentration can be linked, with high velocities being linked to high seston concentrations and low velocities being linked to low seston concentrations. Seston concentrations from side stream sites (Table 3.1) are supportive of this claim as they showed much lower seston concentrations in comparison to main river sites (Figure 4.1) with minimal flows being experienced at these sites. Webster *et al*., (1987), suggest that materials are much larger in side streams and with low velocities to transport these materials, which is why seston concentrations will be lower. Therefore, seston concentrations are dependent on flow velocities, which explains the seasonal trends found during the sampling periods.

pH remained constant throughout the sampling periods (Figure 4.2). Although pH is suggested to influence macro-invertebrate and microbial activities in streams (Tuchman, 1993), the lack of a clear indication that different blackfly species have certain pH thresholds resulted in pH being excluded as a node used for the Bayesian networks (Bns).

Conductivity ($\mu S.cm^{-1}$) showed seasonal variations from the warmer periods to the colder periods. November 2015, March 2016 and December 2016 showed similar conductivities whilst July 2016 showed higher conductivity (Figure 4.3). Highest conductivity was found at the side stream sites and were consistently higher than the main river sites for all four sampling periods. This could be attributed to flow velocities ($m.s^{-1}$), as higher flows have a diluting effect on minerals in the water (Olias *et al*., 2004). This reduces the amount of electrical charges allowed to pass through the water resulting in lower conductivities (Olias *et al*., 2004). When there are low flows, the water is concentrated and therefore high conductivities (Olias *et al*., 2004). Therefore, higher conductivity were associated with lower flows and vice versa. Favourable conductivity ranges found of aquatic life and macro-invertebrates are between 150-500 $\mu S.cm^{-1}$(Horrigan *et al*., 2005), with higher conductivity between and over the range of 800-1000 $\mu S.cm^{-1}$ resulting in a decrease in macro-invertebrate communities (Horrigan *et* al., 2005). This was a factor why the side streams had much lower blackfly larvae and pupae in comparison to the other streams as they had much higher conductivities (Figure 4.6 to 4.9).

Water temperature data (^{o}C) obtained from the HOBO loggers installed at sites recorded seasonal trends as water temperature is directly proportional to ambient temperature (Rutherford *et al*., 1997). Mean water temperatures showed that sites higher upstream and higher in elevation, sites 1 and 2, were cooler than the remainder of the lower stream sites (Figure 4.4). This was expected according to Palmer (1997), as cooler water temperatures are expected in the middle reaches of the Orange River and warmer water temperatures expected in the lower reaches of the river. The only expectations to these were found at sites 8a and 9a, as these side streams had much lower mean temperatures than the main stream sites. Site 9a showed a very wide range in maximum and minimum water temperatures (Figure 4.4). A cooler mean temperature for site 8a than the rest of the main stream sites could have been attributed to shading of the HOBO logger by overhanging trees and vegetation. Whereas, cooler mean temperatures and a wide range at site 9a could possibly be attributed to the HOBO logger being out of the water and the sensor being exposed to the ambient temperature rather than the water temperature. Rutherford *et al.,* (1997) suggests that shading can affect the microclimates of the stream and affect variables such as conduction and water temperature which supports why the side streams (site 8a and 9a) are cooler than the rest of the main stream sites.

5.3. Biotic variations

Blackfly larvae and pupae were collected at all sites during the four sampling periods. *S.chutteri* and *S.damnosum* are the dominant species (Figure 4.5). It was expected that post-dam and impoundment construction, *S.chutteri* would be the dominant species as suggested by various studies along the lower to middle reaches of the Orange River (O'Keeffe and de Moor, 1988; Palmer, 1997; Myburgh and Nevill, 2003; Rivers-Moore *et al*., 2007; Rivers-Moore *et al*., 2014) and this was the case for the November 2015 and March 2016 sampling periods. However, unexpectedly the July 2016 and December 2016 sampling periods were dominated by *S.damnosum*. Emphasis has been placed on *S.damnosum* being the main problem species in western and central Africa (Garms *et al*., 1979; Palmer, 1997; Paugy *et al*., 1999) but not in South Africa.

Palmer and Craig (2000) suggests that *S.damnosum* is more tolerable to lower seston concentrations (mg.ℓ^{-1}) in comparison to *S.chutteri* (Figure 4.7), and this could be a possible factor as to why there was a switching in dominance between the two species, as seston concentrations were observed to be lower in the July 2016 and December 2016 sampling periods. *S.damnosum* is documented from studies in western and central Africa to be a carrier for the nematode *Onchocerca volvulus* which cause the disease commonly known as 'river blindness' amongst humans (Garms *et al*., 1979; Paugy *et al*., 1999; Lamberton *et al.,* 2014), however, South Africa has not had any cases to-date (Palmer, 1997; de Moor, 2003). In South Africa, *S.damnosum* feeds on mammals for a blood meal (Palmer, 1997) and therefore cattle and sheep are equally at risk as they are from *S.chutteri*, which is another factor as to why it was appropriate that these two species were grouped together in the Bayesian networks (Bns) under the complex group strong porous (Palmer and Craig, 2000).

Aside from these two species, the only other species collected that had numbers that could be problematic and could potentially cause an outbreak was *S.adersi*. Historically, this species was dominant along with *S.nigritarse* along the Orange River prior to the construction of the dams and impoundments (O'Keeffe and de Moor, 1988). The economic impacts of an outbreak from this species is not considered as severe in comparison to outbreaks from species belonging to the strong porous complex as *S.adersi* are problematic to birds rather than cattle and sheep (Palmer, 1997). Therefore, livestock would not be affected as much. *S.macmahoni*, *S.nigritarse, S.ruficorne* and *S.impukane* were in low numbers and dismissed as possible species that could cause an outbreak. Therefore, the node *Simulium* within the Bayesian networks (Bns) were based of which three most abundant species for which could be grouped based off Palmer and Craig's (2000) flow velocity and seston concentration model (Figure 4.10). Therefore, the states for the node *Simulium* were that of the species group complex most likely to cause an outbreak as identified from collected species during the four sampling periods, which is why the strong porous and standard complex groups were chosen.

5.4. Spatial resolutions of the Bayesian networks (Bns)

Seston concentration, pH and conductivity displayed no clear evidence of a split in ordination (Figure 4.15 and 4.16), time series water temperature from HOBO loggers at each site showed that the ordination was split into two distinct groupings and a third outlier group consisting of a single site (Figure 4.17 and 4.18). The first grouping of sites consisted of sites 1,2 and 9a. The second grouping consisted of sites 3,4,5,6,7,8,9,10 and 11 and the outlier consisted of site 8a (refer to section 5.2 for why sites experience different water temperatures). The outlier was dismissed as a group that should have a Bayesian network (Bn) constructed for, due this stream was a temporary stream and only one site was in this group. Therefore, it was deemed to be unnecessary for a Bn to be constructed for one site that showed no signs of high abundances of larvae or pupae during any of the four sampling periods. Therefore, spatially the Bns were split in two groups called the upper stream model, for the first grouping of sites, and the lower stream model, for the remainder of the sites. It has been suggested that upper stream water temperatures are generally cooler than that found downstream (Palmer, 1997; Mohseni and Stefan, 1999) which support the splitting of sites. Aside from differing water temperatures, Statzner and Higler (1986), suggests that benthic community structures are different upstream and downstream. Blackfly samples collected at site 1 and 2, which are the upper stream sites, showed less variation in species compared to the downstream sites (Figure 4.6 to 4.9), which is supportive of Statzner and Higler (1986) suggestion of varying community structures upstream and downstream. Nebeker (1971), similarly suggested that altitudinal variation results in different water temperatures that affect macro-invertebrate communities and emergence at different time periods, which was noted by Palmer (1997) along the Orange River with the suggestion made that larvicides could be applied at different times for the lower sections of the river as blackfly emergence is generally later in the upper to middle sections due to cooler water temperatures.

5.5. Data and critical values

Although species thresholds were determined for flow velocity (Figure 4.11), time series data were not available for flow velocity. Return intervals based on the flow velocities collected from the four sampling periods were not sufficient and would result in low accuracy and uncertainties. Thus, critical values for flow velocity were converted to discharge values (Palmer, 1997) and data from Department of Water and Sanitation (DWS) gauging weirs were used to calculate return intervals. Studies by Govers (1992) and Comiti *et al.,* (2007), identified that mean flow velocity can be predicted by discharge and therefore can be assumed that they are linked. This is supportive of the decision to use discharge as opposed to flow velocity as a node within the Bayesian networks (Bns). Although discharge data were not available for each site, which would have been useful for identifying discharge variation amongst sites, return intervals for the node discharge was calculated for each spatial model. Gauging weir D7H002 and D7H005 were deemed the most suitable weir for the upper and lower stream models respectively due to their geographical locations to their respective sites and the historical data available for each model (Table 3.3). However, it must be noted that Palmer (1997) questions the accuracy of many of the gauging weirs along the Orange River as many provide faulty readings. However, discharge data were assumed to be valid. Results suggest that a critical discharge value of 65 $m^3 \cdot s^{-1}$ be used to calculate state probabilities from return intervals calculated from gauging weir data. The same critical value was used by Rivers-Moore *et al*., (2014). The state probabilities calculated for both models showed that most of the time discharge were greater than the critical value (Figure 4.21). The Orange River is characterised by high discharge due to dams and impoundments (Palmer, 1997) and therefore state probabilities obtained were deemed applicable to use within the Bns.

Obtaining a long-term dataset that could be used for seston concentration was a challenge. Atkinson *et al*., (2009) suggests that discharge and seston concentration are linked as observed in studies conducted along the Flint River, of south-western Georgia, USA, where higher discharge conditions existed due to seasonal flooding/rainfall, higher seston concentration was observed. Palmer (1997) suggested that seston concentration and flow could be linked. Therefore, this was why converting discharge values to seston concentration using Palmer's (1997) equation was assumed to be suitable for this study, as discharge values from gauging weirs had a long-term dataset. However, converted seston concentrations from discharge showed a poor relationship with converted seston concentrations from water clarity. Therefore, state probabilities were not calculated from converted discharge data for the seston concentration node within the Bayesian networks (Bns). Seston concentration data, converted from water clarity, were compiled from data collected during the sampling periods and from DAFF monitoring data. Water clarity data were weekly by DAFF at monitoring sites which gave a large enough dataset from which return intervals were calculated for each spatial model. Although the data were not a long-term dataset that was hoped for, continuation of seston concentration data by DAFF during weekly blackfly monitoring at sites will eventually result in a long-term dataset that can be used for updates of the Bns. Seston concentration state populations were improved in comparison to Rivers-Moore *et al*., (2014) methods, which gave assumed values rather than calculated values. The critical seston concentration value, determined from seston concentration (converted from water clarity collected during field visits) against species thresholds, was 25 mg.ℓ^{-1}. Anything above this critical value can be assumed as high seston concentration, and anything below this value can be assumed as low concentration. Palmer and Craig's (2000), supports this as a seston concentration above 25 mg.ℓ^{-1} is suitable for species in the strong porous complex group and in species in the standard complex group. *S.chutteri* are known to favour high seston concentrations, *S.damnosum* favours high to medium seston concentrations. *S.adersi, S.macmahoni* and *S.nigritarse* all favour medium to low seston concentrations (Palmer and Craig, 2000; Rivers-Moore *et al*., 2014).

Although there were no historical water temperature data, data obtained from HOBO loggers were suitable for calculation of return intervals. This was due to a large dataset being obtained as the HOBO loggers collected hourly water temperature data, albeit from a limited period (November 2015 to December 2016). The HOBO loggers were left at each site after the completion of this research, as should future research occur in this system, there will be a long-term dataset on water temperature that can be useful in identifying annual fluctuations. Unlike discharge and seston concentration, water temperature did not have a critical value based on determination of species thresholds from data collected during the sampling periods. The reason for this is that water temperatures during the growth and development of the species may differ from the water temperature that was observed when species were collected. The assumed critical water temperature value of 20 °C was based on a combination of the following nodes; *Simulium*, algae and larvicide efficacy. Therefore, the critical value was a trade-off between these three nodes, as each of these nodes would have a differing threshold of what could be determined as a 'high' or 'low' water temperature state. Palmer (1997), suggests that the pupation of *S.chutteri* larvae is disrupted when the water temperatures are less than 10 °C, with water temperatures greater than 14 °C considered high temperatures. Experiments on *S.chutteri* showed that egg development is more rapid in water temperatures greater than 25 °C in comparison to water temperatures less than 10 °C (Begemann, 1986; Palmer, 1997). For larvicide efficacy, studies showed that temephos was more effective at water temperatures of 20 °C than at 10 °C (Rodrigeus and Kaushik, 1984b; Palmer, 1997). The same effectiveness was noted for *Bacillus thuringiensis var. israelensis* (Bti) with twice the mortality of *S.vittatum* larvae at water temperatures at 20 °C than at 10 °C (Molloy and Jamnback., 1981; Palmer, 1997). Algal blooms are optimal in water temperatures greater than 25 °C (Paerl and Huisman, 2008). With this trade-off, the value of 20 °C would not be the exact or most accurate critical value for any one of the three nodes, but rather a compromise to calculate return intervals for the water temperature node for each spatial model.

The 'parent' nodes 'reeds' and 'spraying' did not have any quantitative data available and therefore return intervals and critical values were not needed or used for these nodes. State probabilities were populated after Conditional Probability Tables (CPTs) were inputted into the Bayesian networks (Bns). Therefore, state probabilities of these two nodes were based on assumptions derived from the CPTs. To improve accuracy for these two nodes, quantification is needed to determine the probabilities of whether reeds are present or absent and whether spraying has been successful or unsuccessful for each spatial model. Potential methods to accomplish these could be through calculating the percentage of in-stream reeds and vegetation and by estimating the mortality rates amongst blackflies when spraying has been applied.

5.6. Conditional Probability Tables (CPTs) population

Each spatial Bayesian network (Bn) had their Conditional Probability Tables (CPTs) populated by quantitative data collected from the field visits. CPTs could have been populated based on expert opinions and values as was done by Rivers-Moore *et al*., (2014), however population of CPTs from quantitative data were deemed more suitable as this method was free from any biasness that could occur with population from expert opinion.

Case files were constructed for each spatial model and was inputted into the Bn through learning algorithms on the software Netica, which was used to construct the Bns. For the upper stream model, there were three sites that fell under this model and therefore its case file was smaller in comparison to the lower stream model which had eight sites. Although the lower stream model has a larger case file than the upper stream model (Appendix 3 and 4), accuracy of the outbreak probabilities generated from the upper stream model should not be considered as less accurate or certain as Bns are known for their capabilities to provide accurate predictions even with small sample sizes (Uusitalo, 2007). A possible critique for when CPTs were populated for each spatial model, was that not all nodes had quantitative data and therefore these nodes had to be assigned assumed states which were based on the state that their 'parent' nodes were observed to be in (Section 3.4.5). Although the literature is supportive of these assumptions, model accuracy and validity would increase should there be quantitative data collected or available for these nodes.

5.7. Generated outbreak probabilities

Blackfly outbreak probabilities generated for each spatial model showed that the upper stream model had a higher probability of an outbreak than the lower stream model. This was unexpected as the upper stream model was based on lower water temperatures than the lower stream model, and higher temperatures are linked to exasperating outbreaks, as higher water temperatures have been linked to a quicker emergence of blackfly adults (Begemann, 1986; Palmer, 1997). A potential reason for this is that the sites that fell under the upper stream model showed less variation in blackfly species collected during the four sampling periods with most of the blackflies collected being from the strong porous complex group (Figure 4.6 to 4.9). The sites falling under the lower stream model, although dominated by the strong porous complex group, showed more variation and a high number of species from the standard complex group (Figure 4.6 to 4.9). Other reasons as to why the upper stream model has a higher outbreak probability could be due to a larger percentage of single channel river being linked to this model in comparison to the lower stream model, which had larger sections of anastomosing river linked with it and therefore greater habitat availability than found in single channel segments. Outbreak probabilities generated showed higher probabilities for the warmer months in comparison to the cooler months for each model (Figure 4.37). This prediction is based on discharge, seston concentration and water temperatures being higher during these seasons along the Orange River.

5.8. Model verification

Although model verification of seasonal outbreak probabilities generated from the Bayesian networks (Bns) differed from blackfly density larvae and pupae scores in terms of most likely and least likely season for an outbreak to occur in (Figure 4.37), statistically the probabilities generated from the Bns and density data showed no significant difference. However, it must be noted that outbreak probabilities generated from the Bns were based on adult emergence. Density scores were based on instream larvae and pupae observed and not the adult emergence. Outbreaks are characteristic of a 'fly worry index', when adult emergence of blackfly swarm and cause annoyance to livestock and humans which result in a loss in quality and productivity (Palmer, 1997; Rivers-Moore *et al*., 2014). Therefore, larvae and pupae do not directly affect livestock or humans, as they are instream suspension feeders (Palmer and Craig, 2000). Outbreaks and annoyance are only felt when larvae and pupae develop into adult blackflies, therefore, there would be a lag time between scored larvae and pupae density and the time that they develop into adults and cause fly worry.

Density scores were expected, as highest blackfly larvae and pupae populations are generally expected in the winter to early spring months (Palmer, 1997), which was comparable to the data collection where the July 2016 samples showed higher numbers (Figure 4.6 to 4.9) and density scores of blackfly larvae and pupae (Figure 4.40). The reason for high densities were due to winter periods have sudden drops water temperatures which can induce larvae and pupae into a state of quiescence (de Moor, 1989). Growth and development are stalled during this period and initiated once water temperatures increase. Adults lay new eggs during this period, which is why larvae and pupae density are highest during winter, however, adult emergence is only expected in spring (de Moor, 1989). The blackfly outbreak probabilities generated from the Bns indicated that summer is the problematic season. The reason for this is due to flow velocities, seston concentration and water temperatures being the highest during this season. Potentially, higher probabilities in summer than in spring could be due to a lack of a biological control node. Larval hydropsychids are absent in late winter to early spring, therefore will not affect blackfly larvae and outbreaks during spring, however, emergence during late spring to early summer of these predators could be a controlling agent of blackfly larvae and pupae (de Moor, 1989). This could result in a lower outbreak probability in summer and higher probability in spring should this node be included in the Bns. However, Palmer (1997) suggests that it is difficult to link larvae abundance to adult emergence and annoyance even though seasonal outbreak probabilities generated from the Bns showed no significant difference with density scores.

Studies conducted by de Beer and Green (2012) indicates monthly blackfly adult annoyance (Figure 5.1) similar to the outbreak probabilities generated from the Bns. de Beer and Green (2012) surveyed farmers along the Orange and Vaal Rivers and asked which months had the highest blackfly annoyances during the year, and it was suggested that the summer months had the highest blackfly annoyance whereas the winter months had the lowest (Figure 5.1). The study by de Beer and Green (2012) is supportive of the outbreak probabilities generated from the Bns and provides confidence in generated outbreak probabilities.

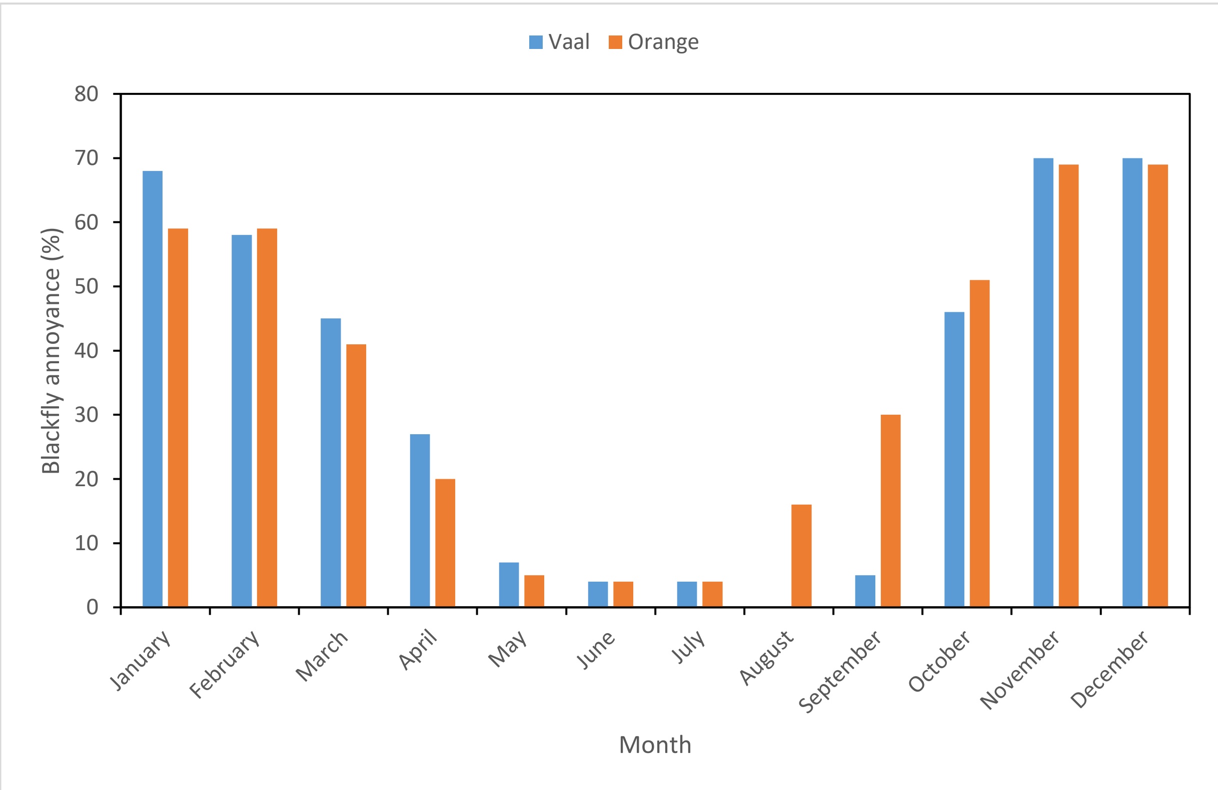

Figure 5.1: Blackfly annoyance indicated by farmers for a specific month along the Orange and Vaal Rivers (de Beer and Green, 2012).

5.9. Scenario analysis

Blackfly outbreak probabilities generated from the Bayesian networks (Bns) under varying scenarios of increase discharge and water temperature, showed that there were increases in probabilities from the baseline values (Figure 4.42). Both spatial models showed that increases in water temperature cause higher outbreak probabilities in comparison to an increase in discharge (Figure 4.42). A study by Ragab and Prudhomme (2002), used global climate models and predicted that the annual average temperatures for southern Africa will increase between 1.5 to 2.5 °C by the year 2050. Wintertime temperatures are expected to increase (Ragab and Prudhomme, 2002) and this will result in water temperatures that will exasperate adult pest blackfly emergence rather than delay it, which is the current case (Palmer, 1997). Therefore, increases in water temperatures will allow for the completion of multiple generations throughout the year, and outbreaks will be more difficult to predict during certain months/seasons (Palmer, 1997). Whilst increases in water temperature will directly influence blackfly adult emergence, increases in discharge will provide suitable habitats in which blackfly growth and development can occur.

Rivers-Moore and Palmer (2017) predicts that there will be an increase in discharge of 60% in the future (2046-2065). Therefore, increases in discharge will further increase habitat availability for pest blackflies such as *S.chutteri* and *S.damnosum*. However, Collier *et al.,* (2008), predicts that southern Africa will become drier and rainfall and discharge will be more variable. Should this occur, blackfly available habitat will reduce which potentially will help improve the blackfly problem faced along the Orange River. The worst-case scenario will be for both water temperatures and discharge to increase, as there will be more available blackfly habitat and more generations of blackfly which will exasperate the outbreak problem (Figure 4.42).

5.10. Reflections and recommendations of the Bayesian networks (Bns)

Prior to this research, blackfly outbreak predictions were based on density scores obtained using Palmer's (1994) rapid ten-point qualitative scoring system, where outbreaks were expected with densities above 7. However, this method is subjective and dependent on whether blackfly larvae and pupae are found in-stream or not, which could be dependent on the person undertaking the monitoring. Thereafter, Rivers-Moore *et al*., (2014) developed a Bayesian network (Bn) to predict blackfly outbreaks. This Bn showed promise however did lack quantitative support in probability and Conditional Probability Tables (CPTs) population. This research refined prediction accuracy from the initial Bn by Rivers-Moore *et al*., (2014) on a spatial and temporal scale, with additions of key nodes and quantitative data to the network. Density data were included in case files used for CPTs. Outbreak probabilities were refined to upper and lower stream Bns, whereas previously outbreak probabilities generated were assumed for the whole river. The generated outbreak probabilities for both models have been verified to produce accurate monthly and seasonal probabilities. These models could potentially be used as a management tool along the middle to low reaches of the Orange River. Management could use the Bns as a support tool for when and where outbreak probabilities are most likely to occur and implement control measures based on this information. Control should be done before the season/month in which high outbreak probabilities are expected, as this will be when high larvae and pupae abundance exists. Multiple Bns along Orange River could result in control methods being done in an optimal and timely way, where higher risk areas could be focused on in comparison to lower risk areas. The Bns could be an engagement tool for other stakeholder affected by blackfly outbreaks, which could result in a holistic control by management and stakeholders. The Bns are able to be updated and therefore should not be considered a once-off model that is just applicable for this research.

Whilst this research incorporated and improved on previous methods to refine blackfly outbreak prediction, further improvements could be made to build upon this research. In addition, an air temperature node could be included in the Bns, as Palmer (1997), suggests that air temperature is a limiting factor in adult blackfly annoyance. A biological control node could be included, and this could help refine outbreak probability for seasons, and spring might have a higher probability than summer. Long term seston concentration data and validation of discharge data could be done to help improve nodes and overall accuracy. The Bns could be adapted into a Bayesian decision network (Bdn) with utility and cost nodes based on management options which could be useful for the blackfly control programme.

5.11. Conclusion

The research provides an account of abiotic and biotic data collected and used for different components of the Bns during all four sampling seasons. The blackfly species complex group most likely to cause an outbreak was noted to be that from the strong porous complex group, and switching of dominance between *S.chutteri* and *S.damnosum* was due to seston concentrations found during sampling periods. The upper stream sites were cooler than the lower stream sites due to geographical conditions, and Bns were constructed for two groups. Outbreak probabilities generated for two spatial models, were both indicative that highest outbreak probabilities are expected during the warmer months in comparison to the colder months. Abiotic variables such as discharge, seston concentration and water temperature were all highest during these months which was why the highest probabilities were predicted for these months.

Chapter Six

Conclusion

6.1. Introduction

The aim of the research was to refine blackfly outbreak probabilities for sites along the middle to lower reaches of the Orange River.

6.2. Objectives

6.2.1. Develop structural components of the Bayesian networks (Bns).

The qualitative component of the Bayesian networks (nodes and links between nodes) was based on Rivers-Moore *et al.,* (2014) conceptual Bn. Additional nodes were added to the Bns which provided greater range and understanding of variables that influence blackfly outbreaks. Nodes were linked via arcs and were either 'parent' or 'child' nodes based on their relationships and dependencies with each other. A maximum of two states were given to each node and no more than three nodes were 'parent' nodes for a 'child' node, as multiple states and 'parent' nodes result in a more complex network.

6.2.2. Identify spatial and temporal resolution for the Bns.

Water temperature data were obtained from HOBO loggers installed at each site which collected hourly water temperatures from November 2015 to December 2016. Water temperature data were used to identify spatial resolution as other abiotic variables collected from the field showed no evidence of a split in ordination, and long-term data for discharge was not available for each site. Two spatial models were derived based on water temperatures and were indicative of an altitudinal variation. Temporally outbreak probabilities from the Bayesian networks (Bns) were generated monthly but then reduced to seasonally as monthly probabilities showed seasonal trends. Refining spatial and temporal resolutions for the Bns were important, as different regions along the Orange River will have different blackfly outbreak probabilities during different seasons. This helped refine accuracy of the middle to lower regions of the Orange River during different seasons, whereas River-Moore *et al*., (2014) had one standard Bn that was applied for the whole river without any temporal resolutions.

6.2.3. Populate the Bns 'parent' and 'child' nodes by calculating probabilities from time series and field data.

'Parent' node had their state probabilities populated when return intervals were calculated from available quantitative data. Critical values were determined from blackfly species thresholds and assumed values (for the water temperature node) and were used to calculate state probabilities from the return interval values for each spatial model. The 'child' nodes probabilities were populated from Conditional Probability Tables (CPTs) that was constructed for each spatial model from data collected and collated into case files during field visits. The previous model by Rivers-Moore *et al*., (2014) had CPTs inputted through expert opinion rather than quantitative case files, therefore imputation through case files led to unbiased and more confidence in generated outbreak probabilities.

6.2.4. Generate blackfly outbreak probabilities under current and potential conditions and verify predicted outbreak probabilities to known outbreak probabilities.

Once 'parent' and 'child' nodes had probabilities and Conditional Probability Tables (CPTs) populated, blackfly outbreak probabilities were generated for each spatial model. Outbreak probabilities were based on current conditions known for discharge, seston concentration and water temperatures. These probabilities were compared to blackfly density data. Although density and Bns had highest outbreak probabilities in different seasons, statistically the seasonal outbreak probabilities were not significantly different. Model verification was not previously undertaken by Rivers-Moore *et al*., (2014), and this process was deemed important as verification lead to confidence in generated outbreak probabilities. Scenario analysis was conducted to show potential outbreak probabilities for scenarios where there potentially is: an increase in water temperature, an increase in discharge, and an increase in both water temperature and discharge. The Bns showed that they could not only be used to generated outbreak probabilities for current data, but additionally could be used to predict probabilities for future scenarios.

6.3. Conclusion

This research fulfilled the set aim and objectives. Bayesian networks (Bns) proved to be a useful and accurate predictor of blackfly outbreaks along the lower to middle reaches of the Orange River, with the highest outbreak probabilities expected in the warmer months in comparison to the cooler months. The Bns used for this research could be modified further to improve accuracy and provide a greater understanding of costs and benefits of management options for the blackfly control programme. However, for this research, the Bns constructed helped refine prediction accuracy and therefore the aim of the research was achieved.

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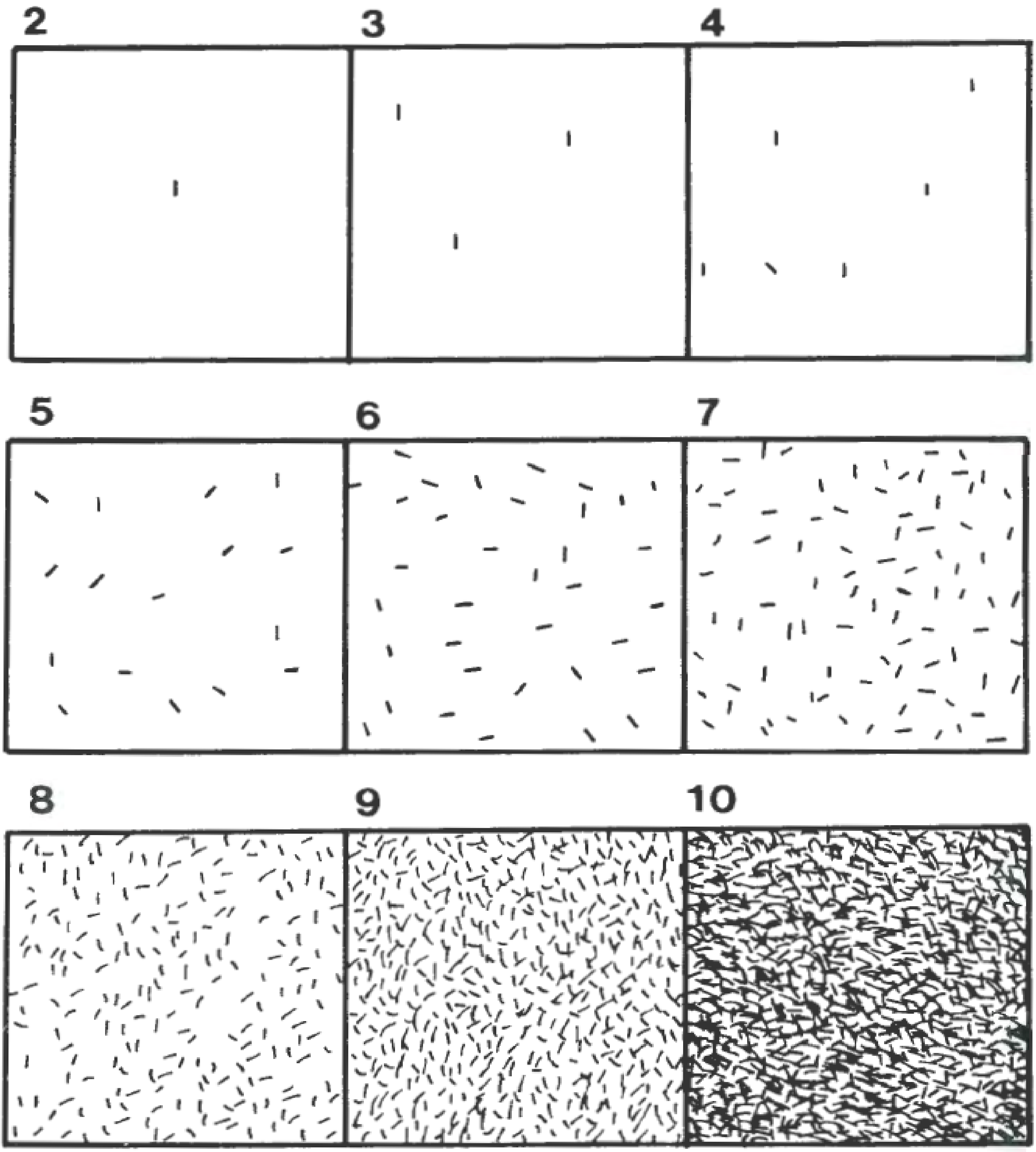
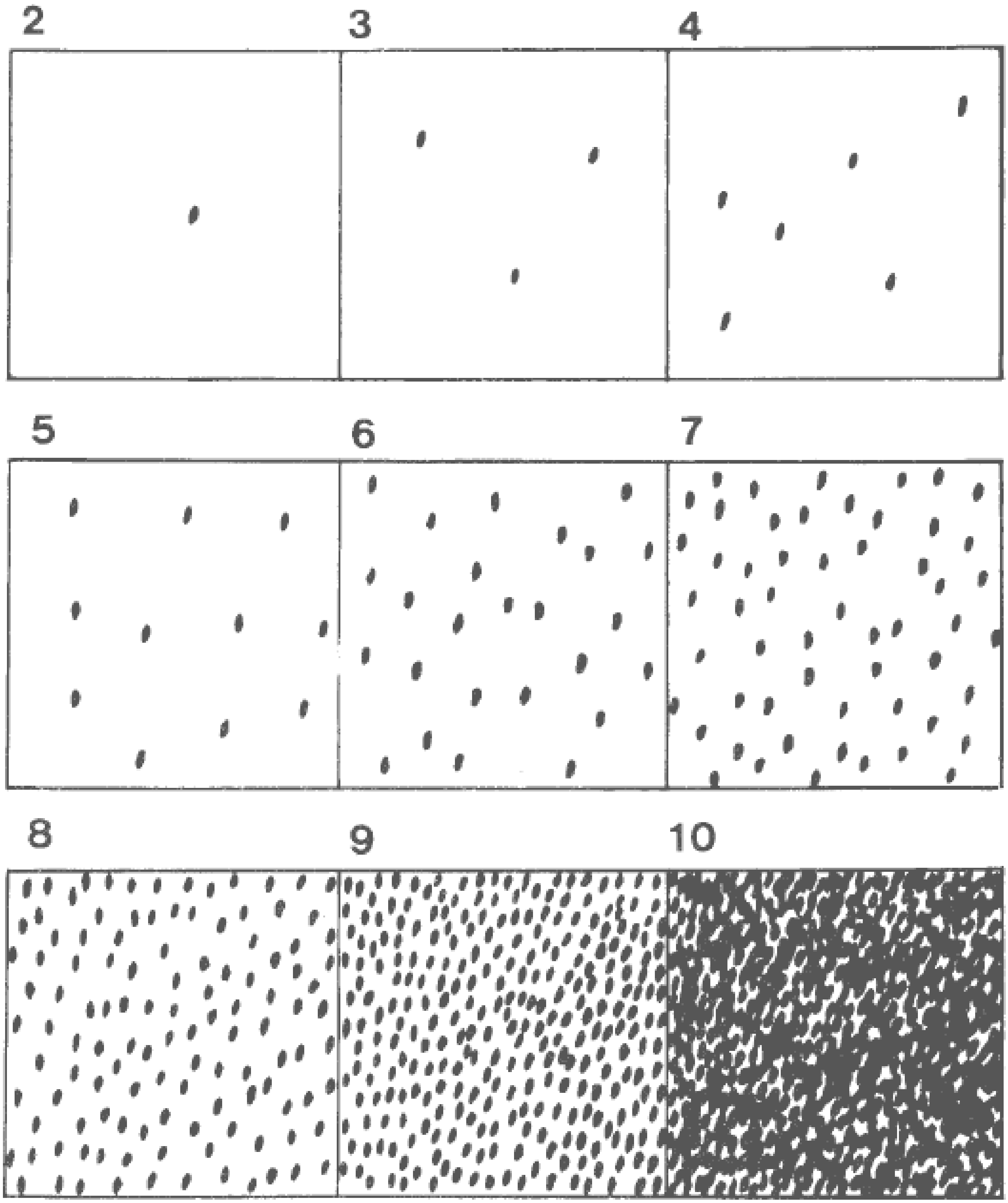
Appendix 1: Table of velocities used for the Transparent Velocity Head Rod (TVHR).

Table of velocities									
ΔH (cm)	Velocity (m/s)	ΔH (cm)	Velocity (m/s)	ΔH (cm)	Velocity (m/s)	ΔH (cm)	Velocity (m/s)	ΔH (cm)	Velocity (m/s)
0.5	0.12	5.5	0.80	10.5	1.17	15.5	1.45	20.5	1.70
1.0	0.24	6.0	0.84	11.0	1.20	16.0	1.48	21.0	1.72
1.5	0.33	6.5	0.88	11.5	1.23	16.5	1.50	21.5	1.74
2.0	0.41	7.0	0.92	12.0	1.26	17.0	1.53	22.0	1.76
2.5	0.48	7.5	0.96	12.5	1.29	17.5	1.55	22.5	1.79
3.0	0.54	8.0	1.00	13.0	1.32	18.0	1.58	23.0	1.81
3.5	0.60	8.5	1.03	13.5	1.34	18.5	1.60	23.5	1.83
4.0	0.65	9.0	1.07	14.0	1.37	19.0	1.63	24.0	1.85
4.5	0.70	9.5	1.10	14.5	1.40	19.5	1.65	24.5	1.87
5.0	0.75	10.0	1.13	15.0	1.43	20.0	1.67	25.0	1.89

Appendix 2: Palmer (1994) scoring chart for blackfly larvae and pupae densities.

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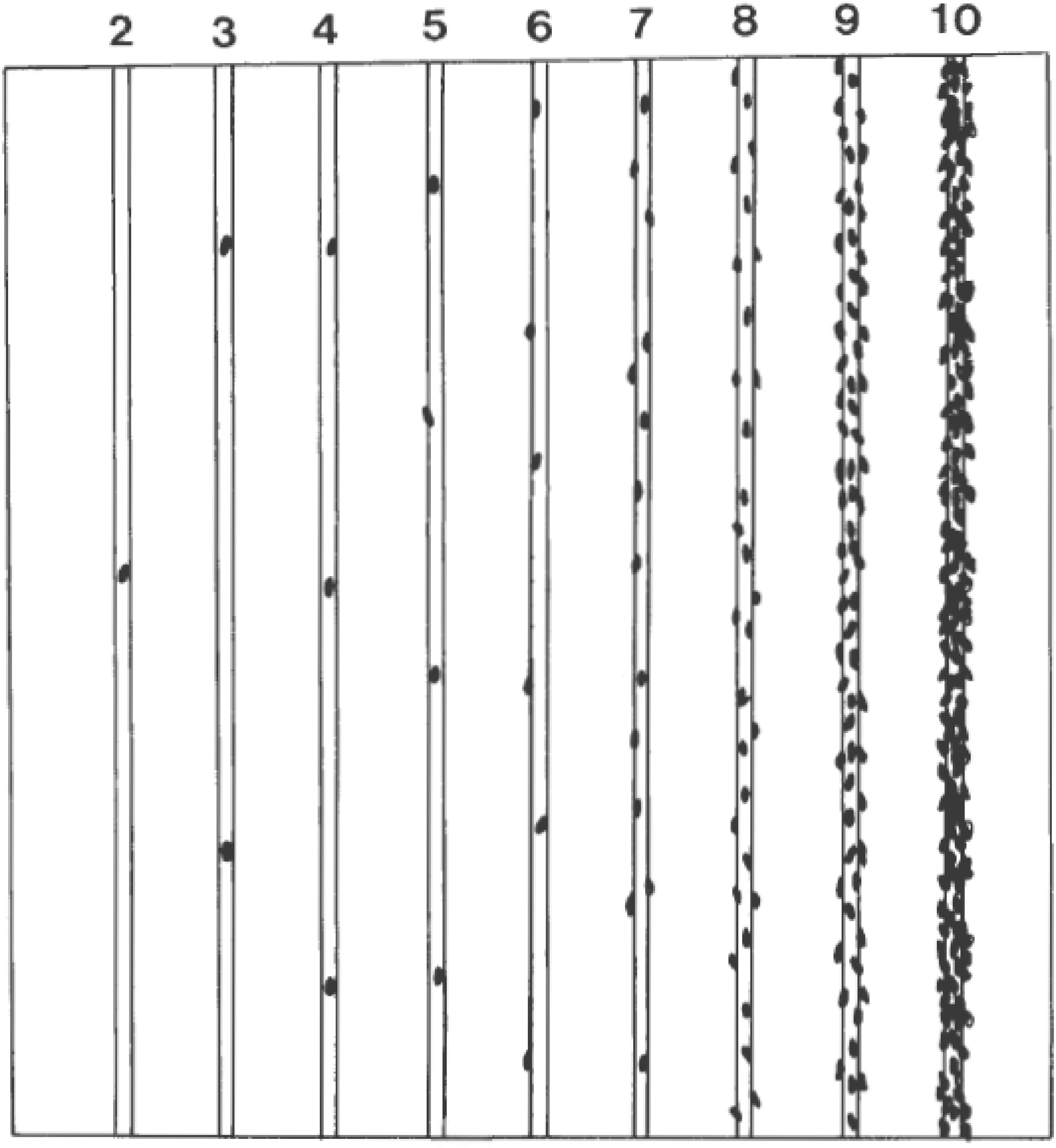

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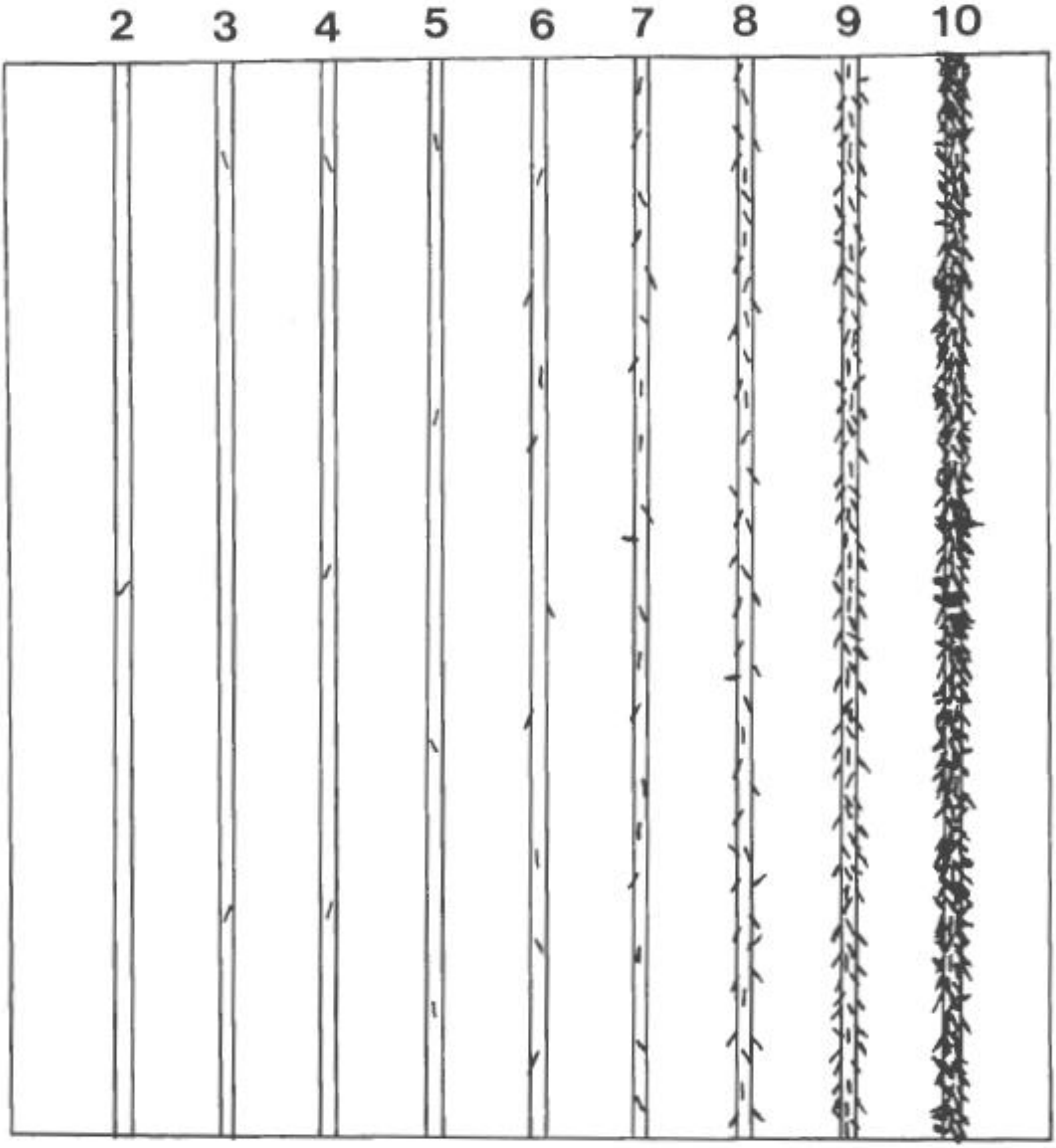
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Appendix 3: Case files for the upper stream model.

Channel Type	Discharge	Seston concentration	Abiotic	Algae	Reeds	Biotic	Larvicide efficacy	Water Temp	Simulium	Management	Spraying	Outbreak Probability
Single	High	High	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High

Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Anastomosing	Low	Low	Unfavourable	Absent	Absent	Weak	Suboptimal	Warm	Standard	Effective	Successful	Low
Anastomosing	Low	Low	Unfavourable	Absent	Absent	Weak	Suboptimal	Warm	Standard	Effective	Successful	Low

Appendix 4: Case files for the lower stream model.

Channel Type	Discharge	Seston concentration	Abiotic	Algae	Reeds	Biotic	Larvicide efficacy	Water Temp	Simulium	Management	Spraying	Outbreak Probability
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	Low	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	Low	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High

Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
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Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
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Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
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Single	Low	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
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Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
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Single	Low	Low	Unfavourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
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Single	Low	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low

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Anastomosing	Low	Low	Unfavourable	Absent	Present	Strong	Optimal	Warm	Standard	Effective	Successful	Low
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Anastomosing	Low	Low	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
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Anastomosing	Low	Low	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Anastomosing	Low	Low	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	High	Low	Unfavourable	Present	Present	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
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Single	High	Low	Favourable	Absent	Present	Strong	Optimal	Warm	Standard	Poor	Unsuccessful	High

Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	Low	Unfavourable	Present	Present	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
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Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Absent	Present	Strong	Suboptimal	Cool	Strong_Porous	Poor	Unsuccessful	High
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Single	Low	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Standard	Effective	Successful	Low
Single	High	High	Favourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	Low	High	Favourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	High	High	Favourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	High	High	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	Low	High	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Effective	Successful	Low
Single	High	High	Favourable	Present	Absent	Weak	Suboptimal	Cool	Standard	Poor	Unsuccessful	High
Single	Low	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Standard	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Standard	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Standard	Poor	Unsuccessful	High
Single	Low	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Standard	Poor	Unsuccessful	High

Single	Low	High	Unfavourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Favourable	Absent	Present	Strong	Optimal	Warm	Strong_Porous	Poor	Successful	High
Single	High	High	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	High	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	High	Unfavourable	Present	Absent	Weak	Suboptimal	Cool	Strong_Porous	Effective	Successful	Low
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	High	Low	Favourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Poor	Unsuccessful	High
Single	Low	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low
Single	Low	High	Unfavourable	Present	Absent	Weak	Optimal	Warm	Strong_Porous	Effective	Successful	Low