INVESTIGATING THE INFLUENCE OF HYDROLOGICAL PHASE ON BAETIDAE AND SIMULIIDAE SPECIES COMPOSITION IN A SOUTH AFRICAN NON-PERENNIAL RIVER: THE SEEKOEI RIVER

by

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DECLARATION

I, **INA S. FERREIRA, 2006012690,** declare that this mini-thesis is my own work, that it has not been submitted for any other degree, at the University of the Free State or any other University or any higher education institution, and that all resources that I have used or quoted are indicated in the text and acknowledged in the list of references.

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Date

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ABSTRACT

All rivers should be monitored to detect changes and disturbances in order to be managed sustainably. Although non-perennial rivers are widespread and common in the semi-arid and arid areas of South Africa they have not been studied extensively. SASS 5 (South African Scoring System version 5) is the standard rapid bio-assessment method used to determine the present state of macroinvertebrates in South African rivers. The SASS 5 method was, however, developed for use in perennial rivers, and regardless of its inaccuracy in non-perennial rivers is still used in these rivers. This study tested the hypothesis that the SASS 5 biomonitoring method does not consider natural changes caused by the hydrology in non-perennial rivers and that family level identification is not accurate enough to reflect the changes in the state of the river. The Seekoei River, used as a case study, is an ephemeral (non-perennial) river, situated in the Northern Cape and is part of the Upper Orange Water Management Area. The autumn samples collected at two sites (EWR 3 and EWR 4; 2006 – 2010) in the Seekoei River during a WRC project (WRC research project K5/1587) were selected for the current study because of the ideal habitat and hydrology experienced at the sites. Two main hydrological phases were identified during the sampling period, i.e. FLOW phase and POOLS phase. Three years (2006, 2008, 2010) experienced the FLOW phase and two years (2007, 2009) the POOLS phase. Two macroinvertebrate families, Simuliidae and Baetidae, were used to determine the influence of species identification on the interpretation of biomonitoring data in non-perennial rivers. The results showed that species within the same family have certain flow and habitat preferences, which would not be detected using family-level data. This should be kept in mind when these rivers are managed. This study concluded that the information available from species-level analysis is important during the management of non-perennial rivers and therefore species-level data together with family-level data should be considered for use.

Keywords: *Seekoei River, non-perennial rivers, SASS 5, biomonitoring, species-level, macroinvertebrates, environmental management.*

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ANOSIM	Analysis of Similarities
ASPT	Average Score per Taxon
CEM	Centre for Environmental Management
EWR	Environmental Water Requirements
GSM	gravel, sand and mud
MDS	Multi Dimensional Scaling
MFV	maximum flow velocity
MIRAI	Macroinvertebrate Response Assessment Index
NoT	Number of Taxa
NWA	National Water Act
PES	Present Ecological Status
PRIMER	Plymouth Routines in Multivariate Ecological Research
SASS 5	South African Scoring System version 5
SD	standard deviation
SIMPER	Similarity Percentages
TDS	Total Dissolved Solids
TWQR	Target Water Quality Range
WRC	Water Research Commission

CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

Environmental management includes the sustainable management of freshwater resources, especially the rivers. By regulation, water use licenses are required for specified water uses (as stated in the National Water Act (NWA); Act 36 of 1998). Before any water license for these specified uses can be issued (or any general authorisation), the Ecological Reserve, in accordance with the NWA (Act 36 of 1998) must first be determined.

The Reserve defined in the NWA is the quality as well as quantity water required for basic human needs, as well as for the protection of South Africa's aquatic ecosystems. Determining the Reserve is only part of an integrated approach known as integrated water resource management and, as illustrated by Pienaar & King (2011), the environmental water requirements (EWR) need to be considered to determine water availability and use. The EWR is defined as the "*water required for maintaining its ecological condition*" (Brown & Louw, 2011). This is only part of the first phase of the process, which will eventually lead to determining the water Reserve and finally the implementation of monitoring programs (part of the adaptive management phase) as illustrated in Pienaar & King (2011).

1.2. RATIONALE

Monitoring systems must be established by the Minister as soon as possible, to assess the quantity and quality of South Africa's water resources (the South African NWA; Act 36 of 1998). Thus, to assess the EWR of a river system, various ecological indicators should be monitored and integrated. The South African Scoring

System version 5 (SASS 5) for macroinvertebrates is one of the methods currently used to monitor the present ecological state of macroinvertebrates in rivers. This method, however, was developed for use in perennial rivers. As non-perennial rivers are highly variable in terms of flow and habitat present, they differ considerably from perennial rivers and therefore methods developed for use in perennial rivers would probably not be ideal. The SASS 5 score obtained during monitoring of a non-perennial river as part of the EWR method could therefore be misinterpreted as it is determined using a method which is considered unsuitable for these systems (Rossouw *et al.*, 2005).

Large areas in South Africa have a semi-arid climate resulting in most rivers being predominantly non-perennial (Davies & Day, 1998). The ecology of non-perennial rivers is more complex than that of perennial rivers, resulting from its unique flow regime (Seaman *et al.*, 2010). It is therefore important to study the ecology of macroinvertebrates in non-perennial rivers as very few data are available. A Water Research Commission (WRC) project (WRC research project K5/1587) regarding the development of a method to determine the EWR of non-perennial rivers found that there is a macroinvertebrate species variance present in the non-perennial Seekoei River, possibly due to hydrological preferences (Seaman *et al.*, 2010). This variance in species is not always visible during normal SASS 5 sampling or interpretation of the results; as macroinvertebrates are only identified to family level in the SASS 5 method. A detailed, taxonomic study will therefore be carried out to determine if the variation in species present during different hydrological phases is statistically relevant.

Water is an important resource and needs to be managed carefully. This study will help develop a better understanding of the macroinvertebrates within non-perennial rivers. A better understanding of the dynamics of macroinvertebrates in non-perennial rivers will most likely contribute to water management in water-scarce areas.

1.3. RESEARCH QUESTION AND OBJECTIVES

1.3.1. The Research Question

How is the species composition of Simuliidae and Baetidae influenced by hydrological phase in the non-perennial Seekoei River and what are the implications for the SASS 5 biomonitoring method?

1.3.2. The Objectives

- To determine the impact of hydrological phase on Baetidae and Simuliidae species composition in a non-perennial river.

- To investigate the implications of change in species composition on biomonitoring and management of a non-perennial river.

CHAPTER 2

LITERATURE REVIEW

2.1. PERENNIAL AND NON-PERENNIAL RIVERS

A perennial river (or perennial stretch of river) is a river which experiences permanent flow throughout the year, every year. This, however, does not mean perennial rivers will never cease to flow, because during severe dry conditions it is possible for non-flow events to occur (Hughes, 2005; Williams, 1987). In order to have permanent flow, these rivers are categorised under one or more of the following descriptions.

- Large rivers, such as the Orange River, that are not as susceptible to drying up due to the size of the water body.
- Rivers which are lower than the surrounding water table are often partially spring-fed (Meinzer, 1923), enabling flow also during the drier periods.
- Rivers found in areas where the precipitation is much higher than the evaporation.

Numerous rivers all over the world, and especially in South Africa, occur within the arid to semi-arid regions where the evaporation exceeds the precipitation. In South Africa most rivers within this dryland area are non-perennial (Davies & Day, 1998). Even though the minority of rivers are perennial, these rivers were studied the most, while research on the non-perennial rivers was neglected due to the complex nature of their systems. This is true not only in South Africa, but internationally (Williams, 1987; Williams, 1988; Davies *et al.*, 1994; Davies *et al.*, 1995).

In water-scarce areas it is extremely important to manage freshwater systems using a sustainable approach, but to successfully manage a river it is also important to understand the functioning of the river ecosystem (Ferreira *et al.*, 2009). The present understanding of river ecosystems is, however, mainly based on perennial systems, just as existing methodologies were mostly developed for perennial rivers. The management of non-perennial rivers therefore needs revision because perennial system-based methods may not be appropriate for use in non-perennial systems (Williams, 1988; Davies *et al.*, 1994).

South Africa is classified as a water-scarce area with growing water demands and this necessitates research on water management in these areas, as it is an important issue which needs to be addressed (Bull & Kirkby, 2002). At present, scientists are beginning to focus their research more on non-perennial rivers, but due to the lack of historical data (especially in terms of the flow regime) this is still a challenge. Research should therefore focus on issues concerning non-perennial systems, such as this study that attempts to better understand the influences of different hydrology on the functioning of non-perennial systems.

2.1.1. Non-perennial Rivers

Non-perennial rivers (often called temporary rivers) are rivers that frequently stop flowing, sometimes for long periods, or completely dry up (Davies & Day, 1998). This river type is abundant and not restricted to South Africa, but widespread all over the dryland areas of the world, especially Australia and Namibia, but also France (Datry, 2012), Italy (Zoppini *et al.*, 2010), Portugal (Aquiloni *et al.*, 2005), Spain (Bonada *et al.*, 2007), Zimbabwe (Chakona *et al.*, 2008) etc.

Although non-perennial rivers are found all over the world, they cannot be treated the same due to the variability from one region to another. The flow regime of Australian rivers is extremely variable due to low mean annual runoff and flood events partially resulting from the high rainfall variability experienced on the continent (Arthington & Pusey, 2003; Finlayson & McMahon, 1988 cited in Lake, 1995). Interesting to note in Australian non-perennial rivers is that, compared to similar rivers elsewhere in the world, these rivers are often colonized by a higher species diversity (Williams, 1987).

Other than the scarce and short flood periods, Namibian non-perennial rivers are dry most of the time or at least without surface water flow, leaving only a few pools in the riverbed (Curtis, 1991). Pools that are spring-fed often support relatively high diversities resulting from the habitat possibilities (slow-flow and pools), but overall the diversity is rather poor (Curtis, 1991; Palmer & Taylor, 2004).

2.1.2. Features distinguishing non-perennial rivers from perennial rivers

The most obvious and probably most important feature that distinguishes these river types from each other is their flow regime. In contrast with perennial rivers, non-perennial rivers do not flow continuously, but instead experience repeated dry phases which vary in time and duration (Davies *et al.*, 1995; Williams, 1987). Various features of these rivers and interaction with their environment result in the drying up of these rivers. Non-perennial rivers are usually smaller rivers/streams present in the dryland areas with the evaporation rates often higher than the precipitation (Davies & Day, 1998).

Fauna present in non-perennial rivers are adapted to survive the harsh conditions characteristic of these ecosystems (Williams, 1987). The trophic structure of non-perennial rivers is dynamic, and changes as the hydrology changes (Rossouw *et al.*, 2005; Watson, 2009). For instance, when the river starts to flow after a completely dry period, the emerging and colonising species change the trophic structure as succession takes place. Also when the rapids between the pools dry up, the species that are dependent on higher flow rates will leave the system or become dormant (Nhiwatiwa *et al.*, 2009; Watson, 2009), also changing the trophic structure. The species composition is thus dynamic, and changes as the hydrology changes (Watson, 2009).

Non-perennial rivers are to a certain extent unpredictable and highly variable systems in terms of flow and habitat availability, leading to a complex ecology (Davies *et al.*, 1995; Seaman *et al.*, 2010; Williams, 1987). This complexity also makes each river different from the other, which leads to the classification of the rivers in order to group those most similar to each other.

2.1.3. Classification of non-perennial rivers

Various classification schemes exist to identify the different types of rivers, of which many are based on some characteristics of the flow regime. Haines *et al.* (1988) did a global classification, while Poff & Ward (1989) did a regional characterisation based on the predictability and variability of streams in the United States. Other scientists have also attempted to describe and group the river types found in South Africa based on the flow records (Joubert & Hurly, 1994; King & Tharme, 1993; Uys & O'Keeffe, 1997)

Many different terms to describe non-perennial rivers were introduced through the various classifications, and temporary, dryland, intermittent, ephemeral, seasonal, interrupted and episodic are some of the commonly used terms (Uys & O'Keeffe, 1997). All the terms imply that the river ceases flow at times, but other than that these terminologies have resulted in some confusion, especially regarding the river dynamics. Uys & O'Keeffe (1997) therefore reviewed the terminology in an attempt to standardise the terms and also proposed the continuum of variability concept. This variability continuum suggests that the flow regimes of rivers change gradually from the most perennial to the most non-perennial.

The current study will use a simplified classification of non-perennial rivers in South Africa, which uses the available historical flow data. The length and severity of the dry periods (thus the climate) can determine the percentage of time that a river experiences no-flow. Rossouw *et al.* (2005) used these percentages (period of no-flow) to classify non-perennial rivers into three categories: semi-permanent (1-25%), ephemeral (26 – 75%) and episodic (>75%). The terminology explained by Uys & O'Keeffe (1997) is used here to define the three categories of Rossouw *et al.* (2005), i.e. semi-permanent (intermittent), ephemeral and episodic (Table 2.1).

Table 2.1: Definitions of intermittent (semi-permanent), ephemeral and episodic rivers as explained in (Uys & O'Keeffe, 1997).

Terms	Definition
Intermittent	Flow stops and some parts in the river may dry for a variable time annually, or during two out of a five year period. Flow can resume seasonally or be highly variable, depending on the climate and rainfall predictability. Several cycles of flow, no-flow and drying can occur over a one year period.
Ephemeral	Flow less time than when the river is dry. Most years during a five year period experience flow or floods for short periods, in response to high and unpredictable rainfall events. Parts of the river support a number of pools.
Episodic	Systems that are highly flashy and flow or flood only as a response to extreme events of rain (often high in its catchment area). Flow may be absent for a five year period or occur only once in several years.

The Seekoei River is identified as an ephemeral river, but seems to reflect some intermittent characteristics according to the definitions in Table 2.1. During intermittent flow, surface water is generally connected but can dry out in certain parts of the river. Ephemeral flow frequently stops, because flow periods are less than no-flow periods, therefore usually only pools remain (surface water disconnected). This then brings us back to the continuum of variability concept of Uys & O'Keeffe (1997). From this concept it is clear that the Seekoei River is an ephemeral river, mostly due to the fact that isolated pools form during drying. The Seekoei River is probably an ephemeral river lying closer to an intermittent river than an episodic river on the continuum; therefore it is important to consider long term flow data when classifying a river.

The flow regime of non-perennial rivers, especially ephemeral rivers, is dynamic and therefore different stages can be identified in terms of the hydrology. These stages are called the hydrological phases and for this study the phases identified by Uys (1997) will be used (Table 2.2). These phases will be used because of its simplicity, thus easy to identify, and were also used by Watson (2009).

Table 2.2: Hydrological phases in non-perennial rivers identified by Uys (1997).

Hydrological Phase	Description
Onset	Period lasting for one month after flow started
Flow	When flow is stable and continuous
No-Flow	The period following flow cessation where surface water is still continuous
Pools	When surface water is discontinuous, thus restricted to pools
Dry	No surface water except in some rainpools

2.2. METHODS TO DETERMINE RIVER HEALTH

2.2.1. Biomonitoring Indices: Aquatic Macroinvertebrates as Indicators

Aquatic macroinvertebrates are considered important bio-indicators for monitoring environmental water quality (Stoian *et al*., 2009). Also, they have been used reasonably successfully all over the world during biological integrity assessments of stream ecosystems (Rosenberg & Resh, 1993; Barbour et al., 1996). According to O'Keeffe & Dickens (2000) this group have been used for monitoring river conditions and its characterisation more often than other biotic groups.

Freshwater macroinvertebrate communities respond rapidly to an impact in the river (positive and negative), and are therefore able to reflect the present condition of a river in terms of the water quality and flow regime (Stoian *et al*., 2009; Thirion, 2007). Macroinvertebrates are ideal to use as indicators because of their diversity (in terms of life history, habitat requirements, and so forth) and their ease of collectability due to small size and immobility (O'Keeffe & Dickens, 2000).

In South Africa, especially because of the lack of identification keys to species level, macroinvertebrate based biomonitoring indices requiring only family level identification were developed (O'Keeffe & Dickens, 2000). These types of methods are usually easier and faster methods, such as the SASS 5 method (Dickens & Graham, 2002).

2.2.2. South African Methods

The SASS 5 method is one of the current methods used to monitor macroinvertebrates when assessing the water requirements in South Africa. The SASS 5 method can be interpreted better when used together with habitat, flow and water quality assessment indices, i.e. the Macroinvertebrate Response Assessment Index (MIRAI).

The South African Scoring System Version 5 (SASS 5):
SASS 5 is the standard method for rapid bio-assessment used in South Africa to assess the health and water quality of a river and works especially well in polluted, perennial rivers (Dickens & Graham, 2002). SASS 5 is intended for rivers with low to moderate flow, and has not yet been tested extensively on non-perennial rivers. Thus, one should use SASS 5 with caution in non-perennial rivers (Dickens & Graham, 2002). In the SASS 5 method three types of biotopes are sampled: stones (in- and out of current); vegetation (marginal and aquatic; in- and out of current); and gravel, sand and mud (GSM). Thereafter the operator takes 15 minutes, per biotope, to identify the organisms to family level by ticking them off on the SASS 5 form. Afterwards the SASS score and Average Score per Taxon (ASPT) are calculated. The SASS score is calculated by summing the 'quality' score (score in terms of pollution resistance/susceptibility) of the families present in the sample, while the ASPT is the SASS score divided by the number of taxa (NoT) identified (Dickens & Graham, 2002). The abundance of each family is estimated and the biotope diversity evaluated.

According to Dickens & Graham (2002) SASS 5 results cannot be used as it is, but should be used together with habitat assessment methods/indices to be significant. SASS 5 is used in studies such as:

- The National River Health Programme;
- The Ecological Reserve determination in rivers;
- Impact assessments (Dickens & Graham, 2002).

The SASS 5 score also does not necessarily reflect the natural changes in non-perennial rivers (Watson & Dallas, 2013). The lower SASS 5 score found in non-perennial rivers, whether during flow or no-flow periods, are mostly interpreted as the result of pollution or degradation (or another impact on the system), thus often seen as a negative effect. The possibility that it could be the result of a natural effect, especially in non-perennial systems, should therefore also be taken into consideration when scores are interpreted (Dickens & Graham, 2002; Seaman *et al.*, 2010). Habitat becomes restricted as flow diminishes naturally during dry periods and this results in a lower SASS 5 score, even during flow periods. The macroinvertebrates that prefer a specific habitat would not be present even though the site experiences flow and does not have any human impacts. In other words, the macroinvertebrates found in non-perennial rivers are generalists because they need to be more adaptable in such systems (Watson & Dallas, 2013). This in turn, results in a lower SASS 5 score because the generalist macroinvertebrates have a lower SASS 5 quality score due to the fact that they are less sensitive to pollution/disturbance.

Macroinvertebrate Response Assessment Index (MIRAI):

The MIRAI is only part of a bigger process, the ecoclassification process, and was developed by Thirion (2007). The method uses the SASS score, invertebrate abundance and presence data, as well as invertebrate preferences in terms of their habitat, water quality and flow when determining the Present Ecological Status (PES) of macroinvertebrates. The result is given as a category, known as the Ecological Category.

The MIRAI also needs a reference site or reference data, because the Ecological Category basically indicates the river condition as a percentage of what it should be (the expected). The reference condition is often difficult to establish, especially for non-perennial rivers. Professional opinions are therefore used to set the reference condition, because of the lack of historical data, making it more difficult and subjective (Watson, 2009). Data extrapolation is also often used to determine reference conditions, but has been proven unreliable especially in non-perennial rivers (Lamprecht, 2009).

<u>Using Different Taxonomic Levels: Family vs. Species:</u>

Using family-level data during rapid biomonitoring methods based on macroinvertebrates in rivers are widely accepted, because it is faster, easier and more cost effective (Marshall *et al.*, 2006). Numerous studies have also proven that the same conclusions can be made using either species- or family-level data (Beketov *et al.*, 2009; Metzeling & Miller, 2001). These studies are often based on the effects of pollution in perennial rivers (e.g.: on European rivers Beketov *et al.*, 2009; on a South African river Ferreira *et al.*, 2009). A study by Metzeling & Miller (2001) on Australian rivers did however find a significant difference between the species- and family-level analyses in the riffles habitat.

Species within the same family can have different flow and water quality preferences (Palmer & de Moor, 1998). If only family-level data is considered, it would not be detected if a specific species have increased, decreased, appeared or disappeared. A case study mentioned in Rossouw (2009) and WCD (2000) on the impacts of the Gariep and Van der kloof Dams indicated that *Simulium chutteri* (Simuliidae) increased drastically and became a pest species in the Orange River as a result of habitat changes due to flow regulation. The same study indicated that *Pseudocloeon vinosum* (Baetidae) disappeared from the river, while the current study and Watson (2009) identified this species in the Seekoei River indicating that non-perennial rivers can act as refugia for species impacted in perennial rivers. Some species will therefore leave the system if conditions changed and especially if this condition persists. If only family-level data is used, important information, as mentioned above, would have been lost.

2.2.3. The Importance of Biomonitoring in Environmental Management

River ecosystems provide essential goods and services, which are needed, directly or indirectly, for the survival and well-being of humanity (Rossouw, 2009). In order for rivers to keep providing these goods and services it is important to protect and manage these ecosystems. The management of freshwater systems, especially rivers, should include the quantity and quality of water, because as a water-scarce country there is a shortage of potable water, not just water (Davies *et al.*, 1995).

Studies have also demonstrated the importance of the habitat and flow regime, and should therefore be included in the management of these resources (Chakona *et al.*, 2008). To be able to successfully manage river ecosystems, changes in river systems resulting from human or natural influences, need to be assessed and measured. To identify any change in a river system the present state needs to be assessed by means of a biomonitoring method (such as the SASS method), and over time the changes will be revealed.

2.3. MACROINVERTEBRATES OF NON-PERENNIAL RIVERS: FACTORS DETERMINING THE PRESENCE OF SPECIFIC MACROINVERTEBRATES

The River Continuum Concept implies that in river systems the community composition of aquatic macroinvertebrates changes longitudinally in terms of their functional group, because of the predictable physical features of rivers (Vannote *et al.*, 1980). The Serial Discontinuity Concept of Ward & Stanford (1983), however, recognises that certain barriers, such as impoundments, form a discontinuum in the river hierarchy and will have varying effects on the river depending on its longitudinal position (Davies & Day, 1998). The species composition is therefore structured according to the habitat, hydrology and water quality, among others.

2.3.1. General

According to Williams (1987) the flow regime does not necessarily influence the diversity, but rather the type of taxa present in temporary systems. That is because macroinvertebrates characteristic to non-perennial rivers tend to be more adapted to harsh conditions specific to non-perennial rivers (Williams, 1987; Watson, 2009). Williams (1987), however, also states that the controlling factors in non-perennial and perennial rivers are equally harsh, due to the fact that perennial rivers are flowing waters. Organisms in perennial systems therefore have adaptations to survive the different flow conditions, while in non-perennial rivers the organisms rather need adaptations to survive the drying periods (no-flow or even no surface water at all). In other words, no matter what river an organism inhabits, there will be certain conditions to which they need to adapt in order to survive and the fact that some need to adapt to lentic and lotic conditions does not make it worse than other conditions.

Various environmental factors contribute to the adaptations reflected in macroinvertebrates, which will determine the species composition in non-perennial rivers namely:

1. Flow regime

   The flow period and range is a major determining factor in a river system's ecology, as it influences most ecological processes (Boulton & Brock, 1999; Williams, 1987). For example, *Paragomphus genei* (Gomphidae) requires at least 70 days of flow to reach maturity (Suhling *et al.*, 2004), thus non-perennial rivers that flow for a shorter period can only support species with faster development times. The presence of some macroinvertebrate species will also depend on the flow range, as some species, such as some Trichorythidae species, are dependent on higher (>0.6m/s) flow rates (Thirion, 2007).

2. Pools

   The formation and duration of the pools (often dependant on the groundwater level and the substrate of the riverbed) will determine what species can survive (Williams, 1987). Pools can be connected or completely isolated from each other. The existence of permanent pools in non-perennial rivers is important because these pools are usually larger or spring-fed, therefore less susceptible to complete dry out. A number of lentic species will be able to survive in a river environment experiencing the pools phase.

3. Water loss and no-flow period

   Non-perennial rivers frequently stop flowing and extreme cases lead to the complete loss of water, thus a dry/terrestrial phase. Aquatic macroinvertebrates in non-perennial rivers are often adapted to survive during no-flow or dry periods, while the majority of macroinvertebrates in perennial systems would die out (Williams, 1987). Depending on their life-cycle, species survive unfavourable conditions as eggs, immature stages or adults (Williams, 1987). Simuliidae species generally need flow for survival therefore during water loss and no-flow conditions such species will be absent (Craig *et al.*, 2012). Macroinvertebrates found in non-perennial rivers tend to overlap with those found in standing waters, because of the no-flow periods (Williams, 1987).

4. Physico-chemical variability

   The physico-chemical parameters of non-perennial rivers can be very unpredictable due to the dynamic flow regime. Macroinvertebrates therefore need the ability to survive in these various water quality conditions or will not be present, thus an important determining factor (Thirion, 2007). These conditions may be anything from low levels of dissolved oxygen, or dramatic changes in pH levels, to a wide range of temperatures (Williams, 1987).

5. Habitat availability

   Habitat availability is a determining factor because different species occupy different niches, for example species composition can vary between different substrates (Minshall & Minshall, 1977). Chakona *et al*. (2008) also indicated that some species prefer the vegetation habitat, while others prefer the cobbles habitat. In other words, the species composition also depends on the quality, quantity and type of habitat present (Louw *et al.*, 2013).

For optimal survival in non-perennial rivers, species often have multiple adaptations in order to deal with a combination of environmental factors (Williams, 1987).

2.3.2. Various adaptations/strategies of macroinvertebrates in non-perennial rivers

The survival of macroinvertebrates in non-perennial rivers mainly depends on their:

1. Physiological adaptations/tolerance
2. Ability to immigrate or emigrate

Species have life-cycles that are adapted to different growth rate patterns and growth periods. The growth period can either be during one or more phases (flow, pools or both), or stretch over one/multiple years. Williams (1987) described different adaptations to the growth patterns demonstrated by different species, which can be summarised as follows: The first adaptation of species is by having one life-cycle stretching over the growth period at an even growth rate throughout. Other species develop very fast in order to have multiple life-cycles in one growth period. Some species hatch immediately after the growth period started and develop rapidly at first, slowing down in the end, often to survive when drying up occurs earlier than usual. In streams with winter-flow, some species are adapted to slow hatching and initial development, speeding up as the water temperature rises. Species may also show a steady growth rate throughout the growth period stretching over one year. Those species with a growth period of two or more years, experience a growth rate peak during the aquatic phases. Thus, species in temporary waters adapt by increasing or decreasing the growth rate of their life-cycle, or part of their life-cycle.

Many macroinvertebrates can escape harsh conditions by moving to the available refugia (Obach *et al*., 2001; Watson, 2009). In non-perennial rivers the marginal vegetation and pools are important refuge areas. These refugia then act as protection against disturbances, such as droughts (Obach *et al*., 2001).

2.3.3. Overview of species recorded in the Seekoei River

It is important to know what species can be present in a certain river and, especially for non-perennial rivers, what their adaptations and preferences are. This information can be very helpful when predicting what species should be present during river assessments. The following table (Table 2.3) provides some adaptations and preferences, including flow preferences, for some of the macroinvertebrate species identified by Watson (2009) as present in the Seekoei River during March 2006. From this table it is demonstrated that, within the same family, the species preferences are different from that of the family. The families Baetidae and Simuliidae are also discussed in more detail as these were the two families chosen as part of this study.

Table 2.3: The flow preferences of some macroinvertebrates recorded in the Seekoei River, with habitat preferences and other adaptations to survive in non-perennial rivers.

Family	Species/ Genus	Hydrological preferences	Habitat preferences/ Adaptations	References
Baetidae	*Cloeon* sp.	No-flow, Slow flow	Present amongst vegetation. Vibrate double gills for sufficient oxygen uptake.	Agnew, 2008; Barber-James & Lugo-Ortiz, 2003
	Pseudocloeon sp.	Various flow	Present under stones and amongst vegetation.	Barber-James & Lugo-Ortiz, 2003
	Nigrobaetis sp.	Fast flow	Present in the riffles area.	Barber-James & Lugo-Ortiz, 2003
	Baetis harrisoni	Various flow	Present under small to medium stones in the riffles.	Barber-James & Lugo-Ortiz, 2003; Lugo-Ortiz *et al.*, 2000
Caenidae	*Caenis capensis*	No-flow, slow flow	Prefer muddy substrates or vegetation.	Barber-James & Lugo-Ortiz, 2003
Leptophlebiidae	All	Flow	Found at rocks, gravel or roots/woody debris along river banks.	Barber-James & Lugo-Ortiz, 2003
	Euthraulus elegans	No-flow, slow flow	Present in stony areas. Able to tolerate no-flow conditions.	Barber-James & Lugo-Ortiz, 2003
Odonata	All	Various flow, no-flow	Prefer the marginal vegetation areas. Sit-and-ambush feeding strategy, but may become active searchers in temporary waters.	Samways & Wilmot, 2003
Libellulidae	All	No-flow, slow flow	Usually present in the shallower water, which is warmer relative to the deeper water. Present on vegetation or muddy substrates	Westfall, Jr., 1987
Belostomatidae	*Appasus capensis*	No-flow, flow	Present in marginal vegetation of flowing rivers. Adults can fly to other water sources in dry periods.	Reavell, 2003
Corixidae	All	No-flow, slow flow	Present in marginal vegetation and in the water column.	Reavell, 2003; Thirion, 2007
Naucoridae	*Laccocoris* sp.	No-flow, flow	At vegetation in pools or stones in streams.	Reavell, 2003
Pleidae	*Plea pullula*	No-flow	Present amongst vegetation.	Reavell, 2003
Hydropsychidae	All	Any flow	Present on stones. Dependant on flow for food –fixed silk retreat consisting of a shelter and net covered with sand-grains, gravel or wood pieces.	De Moor & Scott, 2003
Ecnomidae	*Economus thomasseti*	Any flow	Construct tubular shelters on the underside of stones.	De Moor & Scott, 2003
Ceratopogonidae	*Bezzia* sp.	No-flow, slow flow	Present in marginal vegetation or pools.	De Meillon & Wirth, 2003
Chironomidae	All	No-flow, flow	Present in all river habitats.	Harrison, 2003
	Chironomus sp.	No-flow, slow flow	Prefer a muddy substrate. Tolerance to polluted water. Adapted to survive in low oxygen	Harrison, 2003

Family	Species/ Genus	Hydrological preferences	Habitat preferences/ Adaptations	References
			conditions – haemoglobin assist in respiration.	
	Chironomus pulcher	No-flow, slow flow	Life span is adapted to temporary water bodies by having a shorter larval stage.	Harrison, 2003
	Nanocladius saetheri	Fast flow	Present in the rapids.	Harrison, 2003
Culicidae	*Culex* sp.	No-flow, slow flow	Some species survive long dry periods as adults; laying eggs when conditions get favourable.	Coetzee, 2003
	Anopheles sp.	No-flow, slow flow		Coetzee, 2003
Muscidae	*Lispe* sp.	No-flow, slow flow	Usually present at the marginal areas.	Harrison, Prins & Day, 2003
Simuliidae	*Simulium adersi*	Slow flow to moderate flow	Tolerant of a very wide range of habitats in flowing water. Rapids are often the preferred habitat, especially on partially submerged stones.	de Moor, 2003 Craig & Mary-Sasal, 2013 de Moor, 1982 cited in de Moor *et al.*, 1986 de Moor, 1982 cited in cited in Rivers-Moore *et al.*, 2006
	Simulium chutteri	Fast flow	Pest species with a rapid life-cycle. Need long slow-flow areas above rapids for reproduction.	de Moor, 2003 Louw *et al.*, 2013 de Moor *et al.*, 1986
	Simulium gariepense	Slow flow	Can survive on muddy substrates.	de Moor, 2003
	Simulium damnosum	Fast flow	Tolerant of a wide range of water quality	de Moor, 2003 Palmer & de Moor, 1998
	Simulium hargreavesi	Various flow conditions	Tolerant of a wide range of water quality.	de Moor, 2003 Palmer & de Moor, 1998
	Simulium nigritarse	Slow to fast flow	Tolerant of a wide range of water quality.	de Moor, 2003 Rivers-Moore *et al.*, 2006
	Simulium ruficorne	Slow flow	Are able to survive in no-flow conditions. Tolerant to high salinity conditions.	de Moor, 2003 Louw *et al.* 2013
Dytiscidae	*Laccophilus* sp.	No-flow, flow	Adults and larvae are aquatic. Adults have the ability to fly.	Biström, 2003
Gyrinidae	All	No-flow, slow flow, fast flow	Adults and larvae are aquatic. Adults can swim on the water surface, usually near the edge or marginal vegetation. Adults are able to fly. Larvae consist of tracheal gills for breathing and live at the bottom of the water body.	Stals, 2003

Small minnow mayflies are mayflies categorised in the family Baetidae (Order: Ephemeroptera). Baetidae nymphs are aquatic insects adapted to certain habitats. They can be present in lotic or lentic systems (some even in polluted water) and are often good swimmers, sometimes occurring in high abundances (Bouchard, 2004). Baetidae nymphs are found attaching themselves to vegetation, stones or coarse sand, and while most prefer moderate flowing water some genera occur in still waters (Barber-James & Lugo-Ortiz, 2003; Agnew, 2008). Subtropical Baetidae species experience a very short development stage in the water, which might be an adaptation to species found in non-perennial rivers (Barber-James & Lugo-Ortiz, 2003).

- *Baetis harrisoni* Barnard*:*
  Of all the *Baetis* species present in South Africa, *B. harrisoni* is the most common species (Barber-James & Lugo-Ortiz, 2003). According to Barnard (1932) *B. harrisoni* can be found throughout the year. The nymphs are generally found under stones (small to medium in size) in the riffle areas (Barber-James & Lugo-Ortiz, 2003). Therefore when stones are missing, *B. harrisoni* will probably be absent. This species is also an active swimmer and can be impatient when captured (Barnard, 1932). *B. harrisoni* can be present in a wide range of flow rates from slow to fast (Lugo-Ortiz *et al.*, 2000).

- *Cloeon* Leach:
  Species of this genus, *Cloeon* sp., are well adapted to no-flow conditions by having double gills, which are vibrated in order to get the needed oxygen (Agnew, 2008). *Cloeon* sp. are usually present amongst the vegetation found in river-pools and slow-flow areas (Barber-James & Lugo-Ortiz, 2003). Therefore in non-perennial rivers when only pools are left these species can flourish, but are likely to be found in lower numbers or even absent during moderate to fast flowing systems.

- *Nigrobaetis* Novikova & Kluge:
  Nigrobaetis sp. is present in the riffles with fast-flow of medium-sized rivers (Barber-James & Lugo-Ortiz, 2003).

- *Pseudocloeon* Bengtsson:
  Pseudocloeon sp. can be present in various flow conditions, and occur under stones and amongst vegetation (Barber-James & Lugo-Ortiz, 2003).

Blackflies, also known as buffalo gnats (and many other common names), are small flies belonging to the family Simuliidae (Order: Diptera). This widespread family includes many veterinary, medically and economically important species, such as *Simulium chutteri* and *Simulium adersi*, since the females bite warm blooded animals and humans (Pennak, 1978; Craig & Mary-Sasal, 2013; de Moor, 2003). Only the larval stages and pupae of Simuliidae are aquatic, generally adapted to flow conditions. Simuliidae larvae are predominantly sedentary organisms and thus rely on flow for feeding and respiration. Some species, such as *Simulium ruficorne*, are however adapted to also survive in very low flow and even no-flow conditions (de Moor, 2003).

The larvae have unique morphological adaptations which enables them to attach onto various hard substrates, and thus not be swept away by the current. They consist of an abdominal proleg, bearing numerous hooks arranged in a circle. In order to attach to an object, they spin a patch of silk, attach it to the object and then embed their hooks into the silk (de Moor, 2003). Simuliidae larvae are also well adapted for feeding in flowing water. The cephalic fans on the head capsule are adapted to collect food from the water, which comprise of suspended detritus, such as algae, diatoms and microscopic invertebrates (de Moor, 2003).

After several larval instars the final instar spin a cocoon attached to various substrates and turn into a pupa. Dissolved oxygen is extracted from the water by means of a pair of plastron gills (simple or branched) with its shape typically species-specific. The cocoon shapes vary from open-ended (slower flow) to those closed by a ridge (faster flow) to avoid being washed out of the cocoon (de Moor, 2003).

Simuliidae larvae are often adaptable/tolerant organisms and can be found in a wide variety of habitats and ecological conditions in running water (Palmer & de Moor, 1998; Craig *et al.*, 2012). The high adaptability and tolerance of Simuliidae enable many species to become pests in areas with disturbances, such as impoundment of rivers and water transfer between catchments, changing the flow and habitat of rivers (Palmer & de Moor, 1998).

- *Simulium (Meilloniellum) adersi* (Pomeroy):

  S. adersi is widespread in South Africa. They are present in diverse habitat types, and are therefore considered to be very adaptable and can survive a range of ecological conditions (Craig & Mary-Sasal, 2013; de Moor, 2003; Palmer & de Moor, 1998). This tolerance feature makes *S. adersi* more adapted to the variability and unpredictability of non-perennial rivers. Despite the adaptability and tolerance of *S. adersi*, they do however favour slow-flow conditions according to de Moor (2003) and in some rivers moderate flow rates according to de Moor (1982 cited in Rivers-Moore *et al.*, 2006). In fast-flow conditions *S. adersi* usually occur on vegetation according to Craig & Mary-Sasal (2013). These larvae are able to quickly respond to environmental changes and in some areas this species have been recorded as a pest (Palmer & de Moor, 1998). Palmer & de Moor (1998) also found *S. adersi* can reach high abundances in polluted reaches, turbid rivers and disturbed conditions (close to the outlets of impoundments), but are also found in pristine rivers.

- *Simulium (Edwardsellum) damnosum* s.l. Theobald:

  S. damnosum is a very widespread and common species in South Africa, known as a pest of livestock in certain areas (Palmer & de Moor, 1998). This species is generally present in rivers with fast flow where they attach themselves to stones and vegetation (de Moor, 2003). *S. damnosum* can be abundant in moderate- to slow-flow and disturbed conditions, such as downstream of impoundments, and are tolerant of various water quality conditions (Palmer & de Moor, 1998).

- *Simulium (Afrosimulium) gariepense* de Meillon:

  S. gariepense is the only representative of the subgenus *Afrosimulium* and is regarded endemic to the Orange River Basin (Louw *et al.*, 2013; Palmer & de Moor, 1998). A study on Simuliidae distributions by Palmer & de Moor (1998) indicated that *S. gariepense* have decreased in abundances, and should therefore be considered as worthy of conservation. This species prefer larger, muddy rivers with slow-flow conditions, since they are adapted for feeding in turbid waters thus, also the survival on mud-covered substrates (de Moor, 2003; Palmer & de Moor, 1998).

- *Simulium (Metomphalus) hargreavesi* Gibbins:
  S. hargreavesi can occur in rivers with various flow conditions, and tolerate a wide range of water quality conditions (de Moor, 2003; Palmer & de Moor, 1998). This species is widespread in southern Africa, but appears to be restricted to warm, alkaline rivers (Palmer & de Moor, 1998). According to de Moor (2003) *S. hargreavesi* prefer the stones and vegetation habitats of the rapids areas.

- *Simulium (Nevermannia) nigritarse* Coquillett:
  S. nigritarse is the most widely distributed and common species in southern Africa and have good tolerance to polluted water (de Moor, 2003; Palmer & de Moor, 1998). These organisms are generally found under stones or on vegetation, and are known as very quick colonisers of newly formed streams (Palmer & de Moor, 1998). This is because *S. nigritarse* favours slow to moderate flow conditions, although they can be present in a wide range of flow types (de Moor, 2003). Palmer & de Moor (1998) found *S. nigritarse* to be absent from the Orange River, because they do not occur in large rivers, but they are present in medium-sized and temporary rivers.

- *Simulium (Nevermannia) ruficorne* Macquart:
  S. ruficorne is known as widespread, especially in the drier parts of southern Africa (Louw *et al.*, 2013; Palmer & de Moor, 1998). Like *S. nigritarse*, *S. ruficorne* is a coloniser species, and is therefore typically found in slow-flow and is well adapted to survive in very little flow and even no-flow conditions (de Moor, 2003; Palmer & de Moor, 1998). This is why these organisms are tolerant of high salinity and temperatures (as high as 35°C), which are often associated with no-flow and very slow flow (Louw *et al.*, 2013). *S. ruficorne* is likely to be restricted to temporary waters and is generally found on trailing vegetation, dead leaves, stones and even algae (Palmer & de Moor, 1998).

CHAPTER 3

METHODOLOGY

3.1. THE SEEKOEI RIVER SYSTEM

The Orange River Catchment is the largest catchment in South Africa (ORASECOM, 2011; Swanevelder, 1981) and the Seekoei River is one of the many tributaries flowing into the Orange River. The Seekoei River is located in the Northern Cape and is part of the Upper Orange Water Management Area (Figure 3.1). The Seekoei River falls within the tertiary drainage area D32 with the origin situated about 18km East Southeast of Richmond (31°28'22"S and 24°7'13"E) and flows into the Orange River at Vanderkloof Dam (30°17'22"S and 25°1'7"E) (Figure 3.1). There are a number of tributaries contributing to the flow of the Seekoei River (Figure 3.1), and the Klein Seekoei and Elandskloof are probably the most important tributaries (Seaman *et al.*, 2010).

The climate of the Seekoei River is highly variable and probably the main reason for its complex flow dynamics. The very cold winters (with frequent frost) and hot summers with rainfall (250mm – 400mm per annum) occurring mainly in summer, give an idea of the fluctuating weather conditions (Seaman *et al.*, 2010). The Seekoei River is located within a semi-arid region where the precipitation is lower than the evaporation (Seaman *et al.*, 2010), causing the river to frequently stop flowing. Based on available long-term flow data, Steÿn (2005) calculated that the Seekoei River flows approximately 45 % of the time and according to Rossouw *et al.*, (2005) the Seekoei River can be classified as a non-perennial (ephemeral) river.

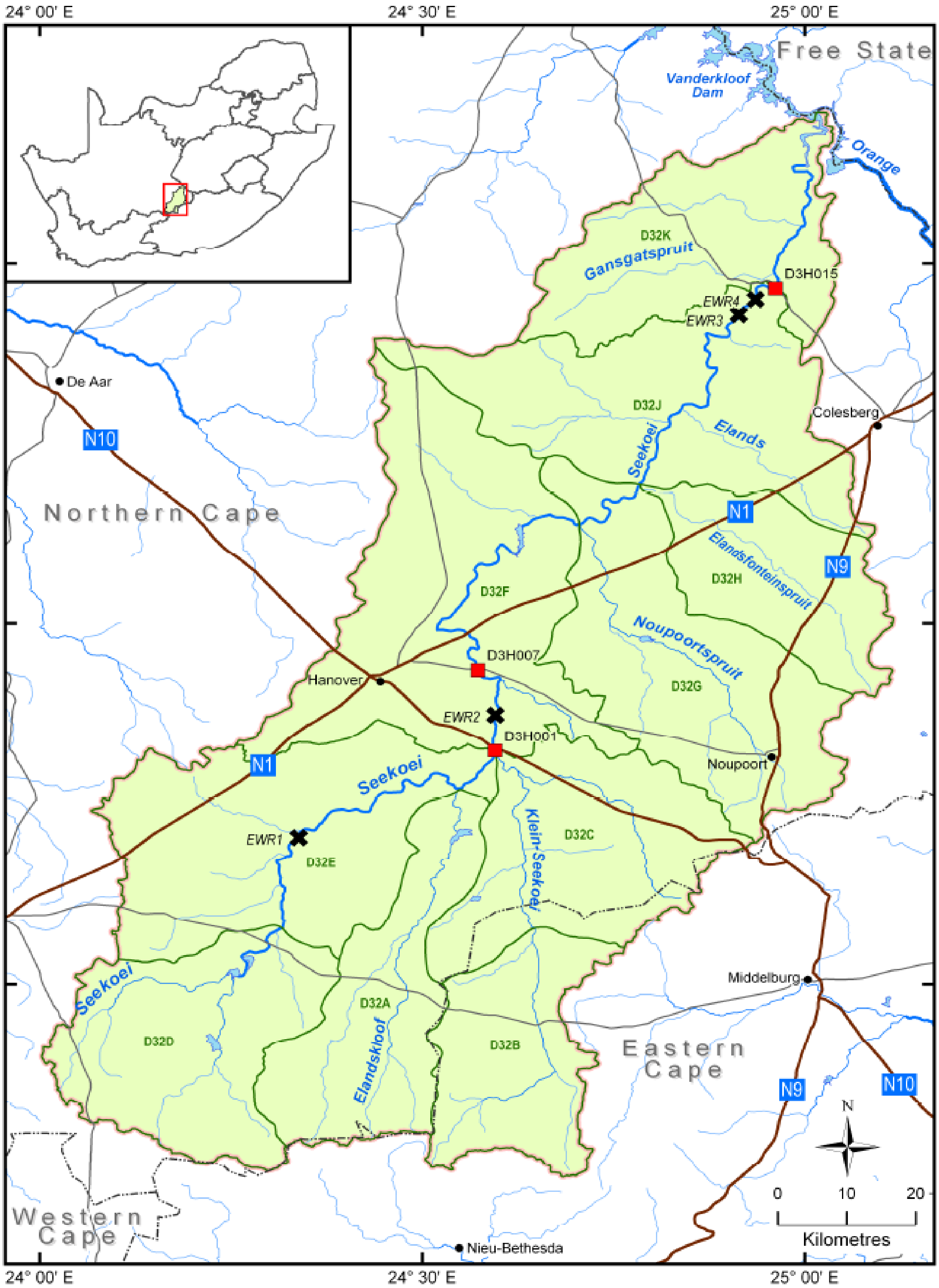

Figure 3.1: The Seekoei River Catchment (quaternary drainage area D32). Indicated on the map are the main tributaries, sites EWR 1 to EWR 4 (black crosses), and the gauging weirs (red blocks) found within the river system. (Data sources: Institute for Water Quality Studies (IWQS), DWA and Chief Directorate of Surveys and Mapping) (Seaman *et al*., 2010).

Extreme droughts and naturally long dry spells cause the river to stop flowing and this leads to the forming of pools, which may eventually dry up. The permanent pools will at least have some moisture throughout time and are believed to provide refuge for biota during droughts, while the non-permanent pools are more likely to dry out completely (Seaman *et al.*, 2010).

The vegetation of the catchment area is identified mainly as part of the Nama Karoo Biome and shrubland is the dominant type of landcover (Mucina & Rutherford, 2006; Watson & Barker, 2006). The landcover is mostly utilised for agricultural (mainly grazing) purposes with only small areas declared for conservation (Rutherford & Westfall, 1994). Activities, such as game farming, stock farming and irrigated crop farming, lead to the construction of numerous dams and weirs used predominantly for irrigation and stock watering (Seaman *et al.*, 2010). According to Watson & Barker (2006) a few smaller towns can be found in the Seekoei River area, therefore some recreational uses also occur along the river system.

All these activities impact on the health of the river to some extent because the overall Instream Habitat Integrity is identified as Class C (Watson & Barker, 2006). This means that moderate modifications to the river habitat exist, especially due to the large number of small dams and weirs within the system.

3.2. MACROINVERTEBRATE SAMPLING

The Centre for Environmental Management (CEM) completed a study on the Seekoei River as part of a WRC project (WRC research project K5/1587; Seaman *et al*., 2010). This research project was an attempt to develop a method for the determination of the EWR of non-perennial rivers. Aquatic macroinvertebrates were collected during field visits at the Seekoei River as part of the WRC project.

Four sites, EWR 1, EWR 2, EWR 3 and EWR 4, were identified at the start of the WRC project for field sampling (Figure 3.1). Aquatic macroinvertebrates were collected 16 times from 2006 to 2010 in the Seekoei River (as specified in Table 3.1).

For the WRC project, the macroinvertebrates were sampled between March 2006 and March 2008, but the CEM continued sampling until 2010.

Table 3.1: A list of the exact sampling dates for the aquatic macroinvertebrates, sampled in the Seekoei River from 2006 to 2010. Field visits marked with the arrow (←) are included as part of this study.

Macroinvertebrate Sampling Dates		
2006 (Site visits occurred about every 6 weeks)	28, 30, 31 March	←
	22-25 May	
	27-29 June	
	15-17 August	
	26-28 September	
	13-15 November	
2007 (Seasonal site visits)	30 January – 1 February	
	20-22 March	←
	12-14 June	
	9-11 October	
During the following years (2008 to 2010) site visits occurred only twice a year: autumn and spring		
2008	25-27 March	←
	5-7 September	
2009	15-17 April	←
	13-15 October	
2010	23-25 March	←
	5-7 October	

The macroinvertebrates were sampled according to the SASS 5 method (Dickens & Graham, 2002). The specific habitat types sampled (Table 3.2), if available, for each of the three biotopes (GSM, Stones and Vegetation) is as follows:

1. GSM:

G	Gravel
S	Sand
M	Mud
Pool	During no-flow periods, pools replaced the GSM biotope

2. Stones:

SIC	Stones-in-current

SOOC Stones-out-of-current

3. Vegetation:

MVIC	Marginal vegetation-in-current
MVOOC	Marginal vegetation-out-of-current
AVIC	Aquatic vegetation-in-current
AVOOC	Aquatic vegetation-out-of-current

The NoT, SASS 5 score and ASPT were calculated for each of the biotopes separately as well as for the combined total per site. The macroinvertebrates collected from the different biotopes in each sample (separated) were then chemically preserved (70% alcohol) to be available for further examination. It is important to note that because the samples were preserved and used for species identification, the results of the microscope analysis cannot be used to calculate the SASS score. The SASS 5 score and ASPT calculated before preservation can however be used as such.

No new fieldwork of any kind was needed as selected samples, collected by the CEM, were used for this study. The selected sampling dates (March/April; 2006-2010, as indicated by arrows) are specified in Table 3.1. Of these samples the following sites and biotopes will be included in this study:

Sites:	EWR 3 and EWR 4
Biotopes:	GSM, Stones and Vegetation

Samples from 2006 – 2010 were chosen for this study, reason being, that this period of five years not only gives the necessary long term data, but it also covers different hydrological phases. Of these samples only the March/April sample of each year (thus one sample per year) was chosen to provide adequate data for this study. A study performed by Steÿn (2005) found that the Seekoei River experienced the highest mean discharge during February and March; therefore flow would more likely occur during these months. If no flow occurred in the Seekoei River at this time of the year, the area most probably experienced a dry year.

Table 3.2: A summary of the different habitat types sampled for each biotope at sites EWR 3 and EWR 4 (March/April; 2006-2010).

Site	Date	Biotope	Available habitat sampled
EWR 3	31/03/2006	GSM	Gravel, Sand
		Stones	SIC, SOOC
		Vegetation	MVIC, AVOOC
	21/03/2007	GSM	Pool (Gravel)
		Vegetation	MVOOC
	26/03/2008	GSM	GSM
		Stones	SIC, SOOC
		Vegetation	MVIC, MVOOC
	16/04/2009	GSM	GSM
		Stones	SOOC
		Vegetation	MVOOC, AVOOC
	24/03/2010	GSM	GSM
		Stones	SIC, SOOC
		Vegetation	MVIC, MVOOC, AVIC, AVOOC
EWR 4	30/03/2006	GSM	Gravel, Sand
		Stones	SIC, SOOC
		Vegetation	MVIC, MVOOC, AVIC, AVOOC
	22/03/2007	GSM	Pool (Gravel, Mud)
		Vegetation	MVOOC
	27/03/2008	GSM	GSM
		Stones	SIC
		Vegetation	MVIC, MVOOC
	17/04/2009	GSM	Pools (GSM)
		Vegetation	MVIC, MVOOC, AVIC, AVOOC
	25/03/2010	GSM	GSM
		Stones	SIC, SOOC
		Vegetation	MVIC, MVOOC, AVIC, AVOOC

3.3. SITES EWR 3 AND EWR 4

Two study sites in the Seekoei River were selected for this study, i.e. EWR 3 (30°34'28.50"S; 24°54'53.28"E) and EWR 4 (30°32'45.5"S; 24°56'08.0"E). Both sites are located at the lower foothills of the farm, Holfontein, and are part of quaternary catchment D32J. Sites EWR 3 and EWR 4 are found in the lower reaches of the

Seekoei River and are upstream of gauging weir D3H015, with site EWR 3 about 2km upstream of site EWR 4 (Figure 3.1). The presence of a weir (Figure 3.2) between site EWR 3 and EWR 4 is responsible for some flow variances between the two sites.

Figure 3.2: The weir located between site EWR 3 and EWR 4 (2011).

Both sites are found in macro-reach 5 of the Seekoei River as identified by Dollar (2005) and are characterised by a steeper topography, with less pools than in the upper reaches. A number of springs are present in the Seekoei River and occur mostly in the lower reaches. The pools in the lower reaches are believed to be dependent on the discharge from these springs (Seaman *et al*., 2010). The pools at sites EWR 3 and EWR 4 are therefore, dependent on the groundwater discharge of the springs located upstream of the sites, especially during times of low or no precipitation.

Sites EWR 3 and EWR 4 were selected mainly because of (A) the hydrological phases experienced during the selected time period, and (B) the available habitat at these two sites. The habitat together with the hydrology makes these two sites ideal to study the species composition of macroinvertebrates.

The hydrology:

The hydrology is probably the most important reason for deciding to study only sites EWR 3 and EWR 4. Sites EWR 1 and EWR 2 consisted mainly of pools during the study period, therefore no hydrological phases could be distinguished. Sampling at these sites did not include the stones (in- or out of current) biotope.

Sites EWR 3 and EWR 4 experienced three main hydrological phases during the sampling period, identified as: FLOW, POOLS and ONSET (see Table 2.2). During the FLOW phase the river experienced moderate to high flow (Maximum flow velocity of 0.48m/s – 1.17m/s), but during the POOLS phase the river formed pools or experienced no-flow conditions. The ONSET phase occurred in March 2008 at site EWR 4 when flow commenced less than four weeks before the time of sampling. This means that some invertebrates, such as Simuliidae, had not established by that time. During the five year study period three years (i.e. 2006, 2008 and 2010) are classified within the FLOW phase, and two years (i.e. 2007 and 2009) are classified within the POOLS phase. From the latter it is clear that the ideal hydrological pattern can be identified, i.e. alternating periods of FLOW and POOLS every year.

The habitat:

The habitat is characterised mainly by pools and rapids (Dollar, 2005). Site EWR 3, as described in Seaman *et al*. (2010), is made up of a large, deep pool and a long rapid/riffle and run (Figure 3.3 – 3.4), with a riverbed of mostly sand in the pool and cobbles and boulders in the rapid/riffle and run. In comparison, site EWR 4 consists of one large, shallow pool and a few rapids and riffles (Figure 3.5 – 3.6); with bedrock dominating the riverbed except for some pools where sand and gravel are dominant.

The biotopes present at sites EWR 3 and EWR 4 are very similar to that found in perennial rivers, as long as there is adequate flow. The three biotopes identified in the SASS 5 method are present at both sites and all are available to sample when there is flow. As the river becomes drier some of the specific habitat types are lost, such as the SIC, MVIC, etc. Often the entire Stones biotope is lost and only pools are left where GSM and MVOOC are the only remaining habitat types (Table 3.2).

The habitat at a site influences the macroinvertebrate community that would be present, especially because of the niches that it provides. Therefore, even though sites EWR 3 and EWR 4 are located near each other, the habitat structure and availability makes each site unique. This variability between sites in the same river, together with the fact that each river also differs from the other, makes non-perennial rivers more difficult to study (Lamprecht, 2009).

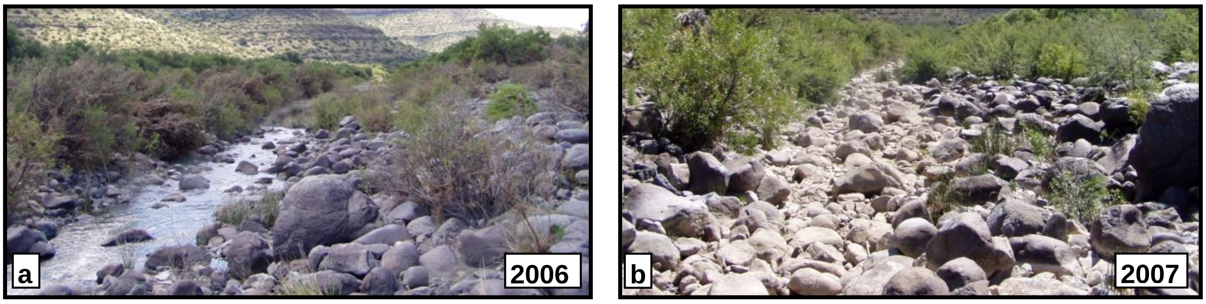

Figure 3.3: The long rapid/riffle at site EWR 3 during a) the FLOW phase and b) the POOLS phase.

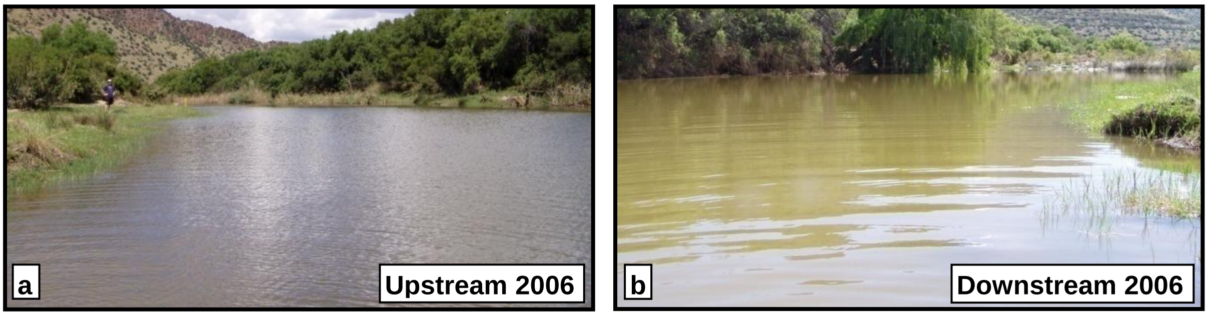

Figure 3.4: The large pool at site EWR 3 showing a) the upstream view and b) the downstream view.

Figure 3.5: Indicating some of the habitat at site EWR 4 (ONSET phase, 2008).

Figure 3.6: The riverbed at site EWR 4 (Pools phase, 2007).

3.4. IDENTIFICATION OF MACROINVERTEBRATE SPECIES

The different biotopes were sampled and preserved separately, which enables analysis between the biotopes. Each site has three samples except in March 2007 (Site EWR3 and EWR4) and April 2009 (Site EWR4) when only two samples were collected. This is because the stones (in-and out-of-current) biotope was absent due to no flow conditions.

In order to help simplify the identification process of the samples certain steps were followed, starting with the cleaning of the samples. The samples were cleaned by separating the macroinvertebrates from the plant material, dirt, etc., using a Carl Zeiss stereomicroscope (10x – 50x) when necessary. The samples from the years 2007 to 2009 were already cleaned, therefore only the six samples collected during March 2010 needed cleaning. All the samples from 2007 up to 2010 were also roughly sorted into families as part of the preparation for the identification.

Originally all the macroinvertebrates were to be identified, but due to a time constraint the study was narrowed down to only two families, i.e. Simuliidae and Baetidae. The macroinvertebrates in the selected samples were identified to species level where possible or at least to genus level. Individuals from the Simuliidae family were identified in the selected samples from 2007 until 2010, but only samples from 2007 were used to identify species in the Baetidae family. Parallel to the identification of the invertebrates, the individuals were also counted to record the abundances of each species.

A Nikon MULTIZOOM AZ100 MULTI-PURPOSE ZOOM MICROSCOPE (10x – 320x) was used to identify the macroinvertebrates as accurately as possible, but no verification was done to ensure the validity of the data. A Nikon camera is connected to the microscope and also to a computer. This was used to help with the identification and to take some photos of the macroinvertebrates (See Appendix A: Figures A1 – A2). Descriptions and keys for the Simuliidae family were used mainly from De Moor (2003), but also from Crosskey (1960) to identify the specimens to species level. Specimens from the family Baetidae were identified using descriptions and keys from Barber-James & Lugo-Ortiz (2003). In some instances the individuals were too small or ruined to such an extent that it was impossible to identify to either species or genus level. These specimens were identified only to family level and the abundances were combined to form the 'unidentified' specimens.

3.5. DATA ANALYSIS

Microsoft Office Excel and Statistica version 12 (developed by Statsoft, Inc. & Microsoft Corporation) were used for the basic analysis, but PRIMER version 6 (Plymouth Routines in Multivariate Ecological Research; developed by Primer-E Ltd) was used for the Multivariate analysis. Most multivariate analysis proved to be of no significant value because of the small size of the data, therefore most will not be included in the results of the study. It was found that Statistica was easier to use for the basic analysis presented in this study, therefore the switch to Statistica.

The gathered species data were entered into a Microsoft Excel spreadsheet. Macroinvertebrate samples from March 2006 at sites EWR 3 and EWR 4 were identified to species level as part of a previous study (Watson, 2009) and were combined with the results from this study. Other data included in the spreadsheet was the hydrological phase, measuring plate depth, physico-chemical data (temperature, conductivity, pH, oxygen, turbidity, TDS), flow data (minimum, maximum and average flow velocity), and SASS data (SASS Score, NoT and ASPT). The monthly rainfall data (South African Weather Services (SAWS)) at Colesberg was entered into a separate Microsoft Excel spreadsheet for analysis.

Various 2D graphs were plotted using the applicable Statistica functions, which include line graphs (different variables over time), box plots (Box-Whiskers), and scatter plots (for correlations). The raw data (e.g. abundance, maximum flow velocity) was used during the analysis, thus no normalisation, standardization or any other form of transformation was applied to the data. The three biotopes were analysed as separate data points within each of the two sites and grouped into the hydrological phases. This enables analysis between sites, biotopes and hydrological phases. The totals per site were also used during some of the analysis.

Line graphs were plotted to see how the different variables (Measuring Plate Depth; Physico-chemical data; Flow data; SASS data; species diversity and abundance for Baetidae, Simuliidae and Baetidae & Simuliidae combined) changed over time. These graphs were only preliminary (See Appendix B, Figure B1, for an example); used for deciding which variables to use and where to go from there. Where data was available for the three biotopes separately, such as the flow data, one graph for each site (EWR 3 and EWR 4) was plotted displaying the biotopes, thus three plots in one graph (Appendix B: Figure B1). If this was not available (e.g. physico-chemical data) the two sites were plotted on the same graph. These graphs (except that of the physico-chemical data) indicated that EWR 3 and EWR 4 demonstrated more or less the same behaviour over time, which is why these two sites will be treated as replicates.

Scatter graphs were plotted to find correlations between some of the variables, such as the abundance or species diversity (number of species present) and the maximum flow velocity, or between the SASS data (e.g. SASS Score against NoT). This was done for Baetidae and Simuliidae separately and combined. During this process it was noted that the abundance data was particularly variable (due to extreme high and low numbers) and that the Baetidae data (thus also the combined data) was very limited. With some exceptions, the diversity data was used during the Statistica analysis.

Box-Whiskers plots were plotted using the box graph function in Statistica. The Box-Whiskers graphs indicate the following: box indicates the 25% to 75% percentile;

whiskers indicate the maximum and minimum non-outlier range; and data points indicate where the median, outliers and extremes are found. This was used to find how the variables differ between the hydrological phases and/or biotopes, with the sites as replicates. The variables included: measuring plate depth, physico-chemical data (temperature, conductivity, pH, oxygen, turbidity and TDS), flow data (minimum, maximum and average flow velocity), SASS data (SASS Score, ASPT and NoT); species diversity data (Baetidae, Simuliidae and Baetidae & Simuliidae combined) and family abundance data (Baetidae and Simuliidae).

Microsoft Office Excel was used to do the basic calculations, including the standard deviation calculated for the physico-chemical data. The rainfall data with the hydrological phases during each identified wet/dry year are illustrated with a combination of the column, line and scatter graphs. The abundance data was also analysed using the Microsoft Office Excel column graphs function. A stacked column graph was created to illustrate the overall abundance of each species for this study, with Simuliidae larvae and pupae separately.

The multivariate analysis included only the Simuliidae abundance data (biotopes and sites separately) of the FLOW phase. From these data only the samples where at least one Simuliidae species were present was selected. The selected data was transformed to presence/absence data with the transform (overall), pre-treatment function in PRIMER. The Bray-Curtis similarity coefficient was calculated between these samples using the Resemblance function. This resemblance matrix was used to run a Bray-Curtis similarity CLUSTER analysis with the group average. A dendrogram was also plotted to visually present the cluster analysis. Groups were then identified at the best similarity (visually decided on the best similarity cut-off) and these groups were also added as a factor.

A Multi Dimensional Scaling (MDS) ordination was presented using the MDS function in PRIMER. The groups identified, with the cut-off similarity, from the cluster analysis are also indicated on the configuration plot with the species contributing most to the similarity (identified with SIMPER analysis).

A one-way Analysis of Similarities (ANOSIM) test was run in PRIMER with the resemblance matrix (used for cluster analysis) to analyse the similarities between the groups identified during the cluster analysis. Note that in PRIMER a 0.1% significance level is equal to $p<0.001\%$ in other programs. This value therefore specifies that there is a <1 in 1000 chance that no difference exist between the groups (Clarke & Warwick, 2001).

The Similarity Percentages (SIMPER) function in PRIMER was used to determine which species contributed the most to the similarity within the groups. The presence/absence data was used to run a one-way SIMPER test of the groups and list only higher contributing variables (cut-off = 90%).

CHAPTER 4

RESULTS AND DISCUSSION SECTION 1: HYDROLOGICAL PHASE AND ITS INFLUENCE ON THE ABIOTIC VARIABLES IN THE SEEKOEI RIVER

4.1. HYDROLOGICAL PHASES OF THE SEEKOEI RIVER

To test for variations in the macroinvertebrate species composition due to changes in the flow phases, it is important to establish the hydrological phases, present in the Seekoei River, which would be applicable to this study. Figure 4.1 combines different aspects, such as total and monthly rainfall, in an attempt to explain which hydrological phase is applicable in each of the identified wet/dry years.

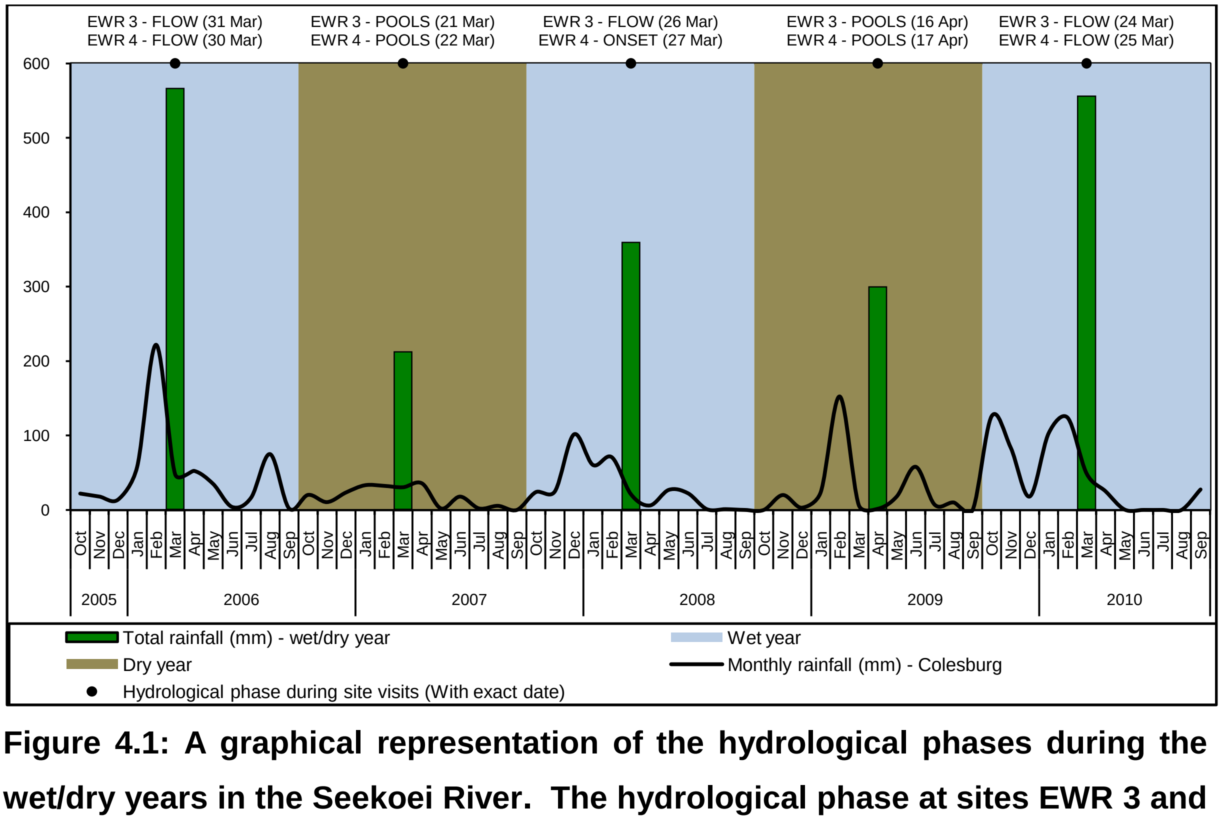

Figure 4.1: A graphical representation of the hydrological phases during the wet/dry years in the Seekoei River. The hydrological phase at sites EWR 3 and EWR 4 during the March/April site visits are provided in the data labels at the top of the graph.

The daily rainfall data at Colesberg (Station [0172164 1]) was supplied by the SAWS for October 2005 to September 2010. Using Microsoft Excel, the monthly rainfall data was calculated and plotted as a scattered chart type with smooth lines (represented by the black line; Figure 4.1).

A wet/dry year, for this project, stretches from October to September of the next year, which positions the March/April site visits roughly in the middle of each wet/dry year cycle. The total yearly rainfall, calculated for each wet/dry year, was plotted as a column chart type in the month of sampling (represented by the green bars; Figure 4.1). The total rainfall clearly indicates that during the dry years the total rainfall was lower than during the wet years.

The hydrological phase experienced during each March/April site visit is also indicated on the graph (see Figure 4.1) together with the exact date of each site visit. This information indicates that during a dry year both sites, EWR 3 and EWR 4, experienced the POOLS hydrological phase at the time of sampling. During the wet year, however, the sites either experienced the FLOW or the ONSET hydrological phase at the time of sampling. In this study a wet year therefore, represents the FLOW phase and a dry year the POOLS phase, but during a wet year a specific site could also experience an ONSET phase.

Although a river experiences flow during both the ONSET and FLOW phase, major differences do exist. Some important differences are demonstrated in various correlations between the number of Simuliidae species and the maximum flow velocity (See Figures B2 – B4, Appendix B for graphs). Three correlations were plotted, one for each of the above mentioned phases. The following observations were made from these correlations: 1) During the FLOW phase there was flow and Simuliidae species present; 2) During the POOLS phase there was neither flow nor Simuliidae species present; and 3) During the ONSET phase there was flow but no Simuliidae species were present. The fact that there were no Simuliidae species present distinguishes the ONSET phase, where there was flow, from the FLOW and POOLS phases.

These basic observations lead to the decision that the ONSET phase should be treated as a separate hydrological phase and cannot be merged with the FLOW phase. Three hydrological phases were therefore identified as applicable to this study:

- FLOW phase
- POOLS phase
- ONSET phase

4.2. ABIOTIC FACTORS INFLUENCED BY THE HYDROLOGICAL PHASES IN THE SEEKOEI RIVER

This section deals with how the measuring plate depth, physico-chemical variables and maximum flow velocity of the Seekoei River changed in terms of the hydrological phase.

4.2.1. Measuring Plate Depth

The measuring plate depth is not an accurate measurement of the river's depth, but adequately indicates an increase or decrease in the water level. The measuring plates were placed in the pool area of both sites in the Seekoei River (Figure 4.2), because when flow ceases the pools are more likely to hold water during dry conditions.

Figure 4.3 presents the measuring plate depth by means of two superimposed graphs, i.e. a bar graph and a box-whiskers graph. The bar graph provides the measuring plate depth recorded at each site visit from 2006 – 2010. This shows how the water level changed over time and indicates that sites EWR 3 and EWR 4 are very similar to each other.

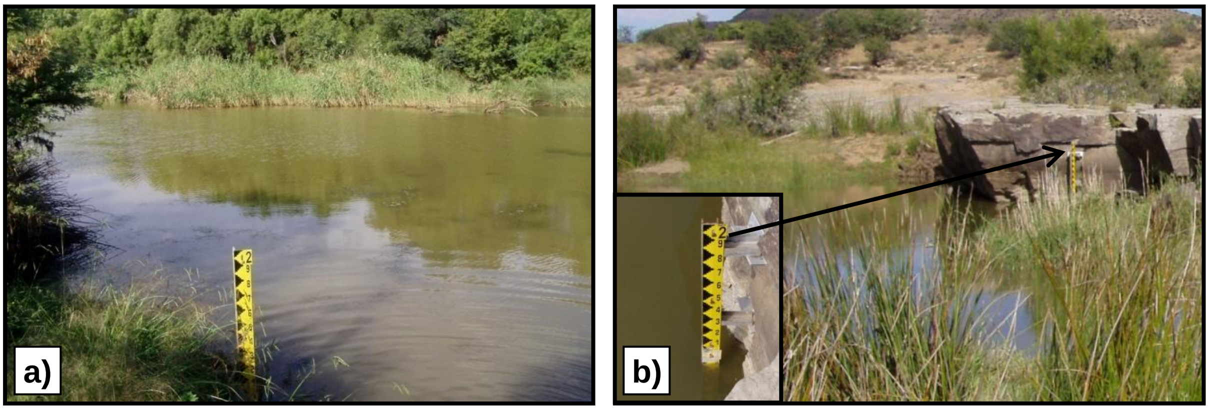

Figure 4.2: The measuring plates located in the major pools of each site, a) Site EWR 3 and b) Site EWR 4 with an enlargement of the measuring plate.

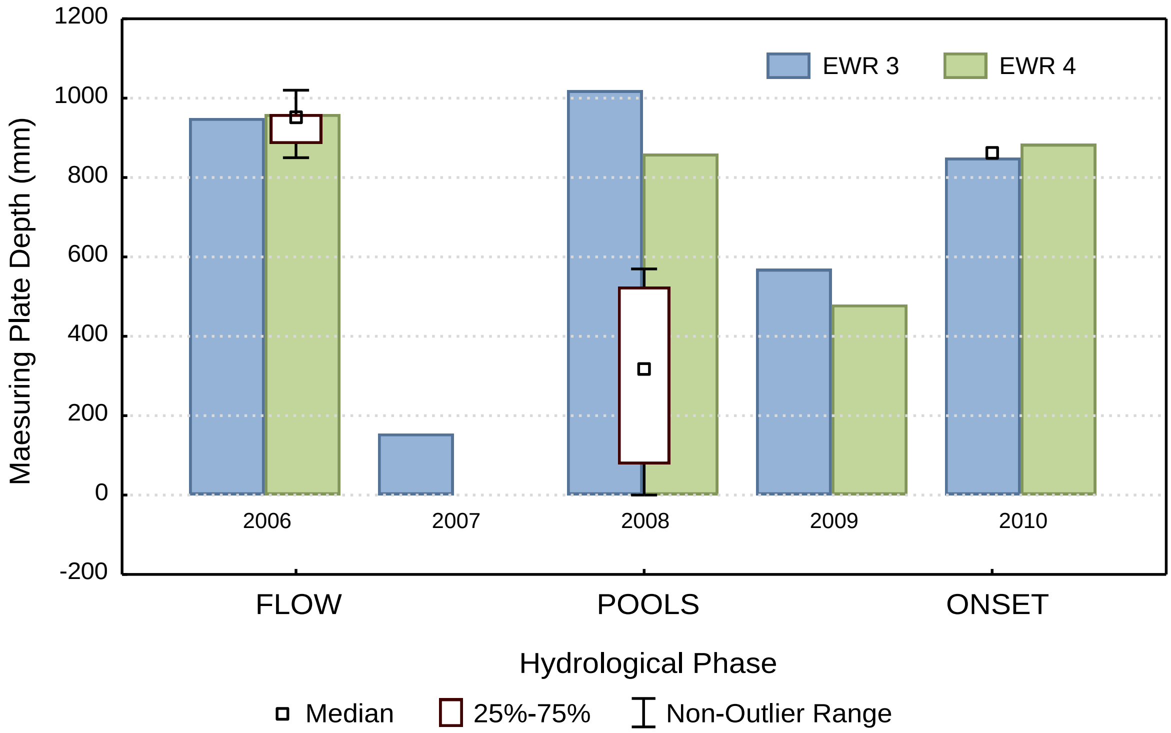

Figure 4.3: The measuring plate depth at sites EWR 3 and EWR 4 in the Seekoei River. The bar graph (secondary x-axis – years) represents the depth recorded during site visits (sites indicated in top right legend). The superimposed box-whiskers graph (primary x-axis) groups the depth into three hydrological phases, i.e. POOLS, FLOW and ONSET (legend at bottom of graph).

The box-whiskers plot groups the data into the three hydrological phases: FLOW, POOLS and ONSET. This clearly indicates that the water level differs significantly between the POOLS phase and the FLOW/ONSET phase. During the POOLS phase (site visits in 2007 and 2009) the water level was below 600mm. The lowest water level was measured as 0mm, at site EWR 4, March 2007 (POOLS phase). This is only because the water level in the pool was so low that it did not reach the measuring plate, as indicated by Figure 4.4.

Figure 4.4: The measuring plate at site EWR4 during March 2007, indicating that, although it was dry at the measuring plate there was indeed water in the pool.

The water level during the FLOW phase was higher, with measurements always above 800mm and a maximum water level of 1020mm (site EWR 3, March 2008). Only site EWR 4 during March 2008 experienced the ONSET phase, thus only one measurement of 860mm is available for this phase. The current study will therefore only focus on the two main hydrological phases: 1) the POOLS phase and 2) the FLOW phase, because there is not sufficient data for the ONSET phase. Unless specifically referred to, data on the ONSET phase was not included in the results.

4.2.2. Physico-chemical Data

The physico-chemical data given in Table 4.1 was recorded at sites EWR 3 and EWR 4 in the Seekoei River, from 2006 to 2010 (during the March/April site visits). This data is inadequate for accurate differences between the two phases, because only one instantaneous reading was taken per site during each site visit. These measurements were always taken in the pool area and more or less at the same time and same place (or at least as close as possible, as these areas are dependent on water level changes). Not only can these physico-chemical factors influence each other, but many other factors (abiotic and biotic) can have an influence on the physico-chemical data (DWAF, 1996). This study will therefore, only consider the possible influences of the hydrological changes on the physico-chemical data in the Seekoei River. Only certain variables, which include the temperature, conductivity, pH, dissolved oxygen, turbidity and TDS, will be dealt with briefly in this section.

Table 4.1: The physico-chemical results for site EWR 3 and EWR 4, taken from 2006 until 2010 during the March/April site visits, with the standard deviation for each variable.

Year	Temperature (°C)		Conductivity (mS/m)		pH		Oxygen mg/l		Oxygen %		Turbidity (NTU)		TDS (mg/l)	
	EWR3	EWR4	EWR3	EWR4	EWR3	EWR4	EWR3	EWR4	EWR3	EWR4	EWR3	EWR4	EWR3	EWR4
FLOW Phase and ONSET Phase														
2006	19.9	20.2	61.4	50.5	8.24	8.44	3.44	7	38	78.7	5.6	25	450.57	369.52
2008	19.7	20.7	60.2	50.6	8.32	8.27	4.59	3.96	51.6	44.3	4.1	31	434.15	373.88
2010	20.5	20.6	33.7	39.09	7.32	7.72	3.44	3.88	38.7	43.5	31	36	243.38	309.8
SD	0.403		11.099		0.436		1.349		15.285		13.830		77.497	
POOLS Phase														
2007	18.5	20.5	97.9	183.1	9.35	9.64	6.16	5.72	67.8	64.3	20	39	790.8	1102.72
2009	18.5	17.1	58.8	62.3	7.51	8.86	3.66	4.99	39.4	51.6	12.6	8.8	446.54	556.64
SD	1.399		57.815		0.943		1.094		12.947		13.431		290.336	

Note: Data are separated into FLOW/ONSET phase (wet years) and POOLS phase (dry years). The data from the ONSET phase are underlined. Variables included are: temperature (°C), conductivity (mS/m), pH, oxygen (mg/l and %), turbidity (NTU; Nephelometric Turbidity Units) and total dissolved solids (TDS).

Temperature:

The temperature during the FLOW/ONSET phase ranged from 19.7°C to 20.7°C, thus having a standard deviation (SD) of 0.403. The POOLS phase on the other hand had a wider range (min. 17.1°C; max. 20.5°C), with a SD of 1.399. Closer examination shows that all but one reading in the POOLS phase was lower than the minimum temperature for the FLOW phase. The exceptionally high temperature during the POOLS phase was measured as 20.5°C at site EWR 4 in March 2007, when the measuring plate depth was recorded as 0mm.

The POOLS phase had a higher standard deviation than the FLOW/ONSET phase, implying that in the Seekoei River the temperature of standing water can be more variable than flowing water over long periods. Based on this data, larger standing water bodies appear to have lower temperatures than small, shallow standing water bodies in the Seekoei River.

Conductivity (mS/m):

The conductivity measured from 2006 to 2010 in the Seekoei River ranged from 33.7mS/m to 183.1mS/m. The conductivity in the POOLS phase was more variable (SD of 57.815) than during the FLOW/ONSET phase (SD of 11.099) and increased with very low water levels in the POOLS phase (during the 2007 site visits).

In general the conductivity tends to be higher in small shallow pools with no flow conditions, while mostly lower values were recorded during flow conditions. Based on this data the conductivity measured in the Seekoei River can have a wider range in standing water over long periods.

pH:

The pH was generally less variable in the FLOW/ONSET phase (SD of 0.436) than in the POOLS phase (SD of 0.943). The minimum pH value (7.32) was recorded during the FLOW phase (EWR 3, 2010) while the maximum (9.64) was measured during the lowest water level in the POOLS phase (EWR 4, 2007).

Based on this data the pH value had a wider range for standing waters in the Seekoei River, and the highest pH values were measured during the lowest water levels (EWR 3 and EWR 4, 2007).

Dissolved Oxygen:

The dissolved oxygen is somewhat more variable in the FLOW/ONSET phase, with a SD of 1.349, compared to the SD of 1.094 for the POOLS phase. Both the minimum (3.4mg/l; site EWR 3 in 2006 and 2010) and the maximum (7mg/l; 2006 at site EWR 4) were recorded during the FLOW/ONSET phase.

Based on this data there seems to be no significant differences between the POOLS phase and the FLOW/ONSET phase in the Seekoei River. The dissolved oxygen levels in the Seekoei River are, therefore, very unpredictable in terms of the hydrological phases, as many other factors can cause a decrease or increase in the dissolved oxygen concentrations (DWAF, 1996).

Turbidity:

The turbidity, just as the dissolved oxygen, seems to be very unpredictable in terms of the hydrological phases in the Seekoei River. The highest turbidity (39NTU) was measured during the POOLS phase in 2007 at site EWR 4. The lowest turbidity (4.1NTU) was recorded at site EWR 3 (2008) during the FLOW phase. The POOLS phase was somewhat less variable with a SD of 13.431, compared to the SD of 13.830 during the FLOW/ONSET phase.

Total Dissolved Solids (TDS):

During the FLOW/ONSET phase the TDS ranged from 243.38mg/l to 450.57mg/l, with a SD of 77.497. A SD of 290.336 indicates that the POOLS phase was more variable with a TDS ranging from 446.54mg/l to 1102.72mg/l.

Based on the given data it seems that in the Seekoei River the TDS levels tend to be relatively higher and more variable in the standing waters of the POOLS phase.

Summary:

There are no clear differences in the water quality of the Seekoei River between the FLOW/ONSET phase and the POOLS phase in terms of the physico-chemical data. It does, however seem that the physico-chemical variables are more variable during the POOLS phase than the FLOW phase, especially in terms of the temperature, conductivity, pH and TDS. In Zimbabwe, Nhiwatiwa *et al.* (2009) also recorded the temperature, conductivity and pH to be more variable at the pool sites compared to the river sites

Although macroinvertebrates in non-perennial rivers are adapted to wider ranges, it is the duration, amplitude and frequency of changes in the natural cycles that might disrupt certain functions, whether physiological or ecological, of the aquatic macroinvertebrates (DWAF, 1996). In order to compare the physico-chemical data to the Target Water Quality Range (TWQR), hourly measurements over a 24 hour period at least, are needed as well as background data on the natural cycles (DWAF, 1996)

4.2.3. Maximum Flow Velocity in the Seekoei River

Various flow velocity measurements were recorded for each site during site visits. For this study only the maximum flow velocity in each biotope at each site during site visits are used, thus three maximum flow velocity values per site, per year. To avoid confusion this will be referred to as MFV, therefore when referring to maximum it means the highest MFV for that particular range.

MFV of the hydrological phases: FLOW phase vs. POOLS phase:
The MFV measured in the Seekoei River indicates that flow only occurred during the FLOW phases, with no flow during the POOLS phases (Median: 0m/s), as would be expected. The FLOW phase had variable MFV throughout the study period with the median equal to 0.54m/s. The maximum MFV rates for each site visit during the FLOW phases varied from 0.48m/s (Site EWR 3, 2010) to 1.17m/s (Site EWR 3, 2008).

Flow variability between the biotopes: GSM, Stones and Vegetation:
The POOLS phase experienced no flow throughout the study duration, therefore only the FLOW phase is considered during this section. The MFV of the three biotopes (GSM, Vegetation and Stones) recorded during each site visit, clearly differs from each other (Figure 4.5).

The GSM biotope experienced flow only during March 2010 at site EWR 4 when a MFV of 0.39m/s occurred (Extreme point – Figure 4.5). This was higher than the maximum MFV (0.34m/s) measured in the Vegetation biotope at the same site and year. It is also possible that there was extremely low flow when a measurement of 0m/s was recorded in the GSM biotope, especially during the FLOW phase as the flow was probably just too low for the flow meter to record it.

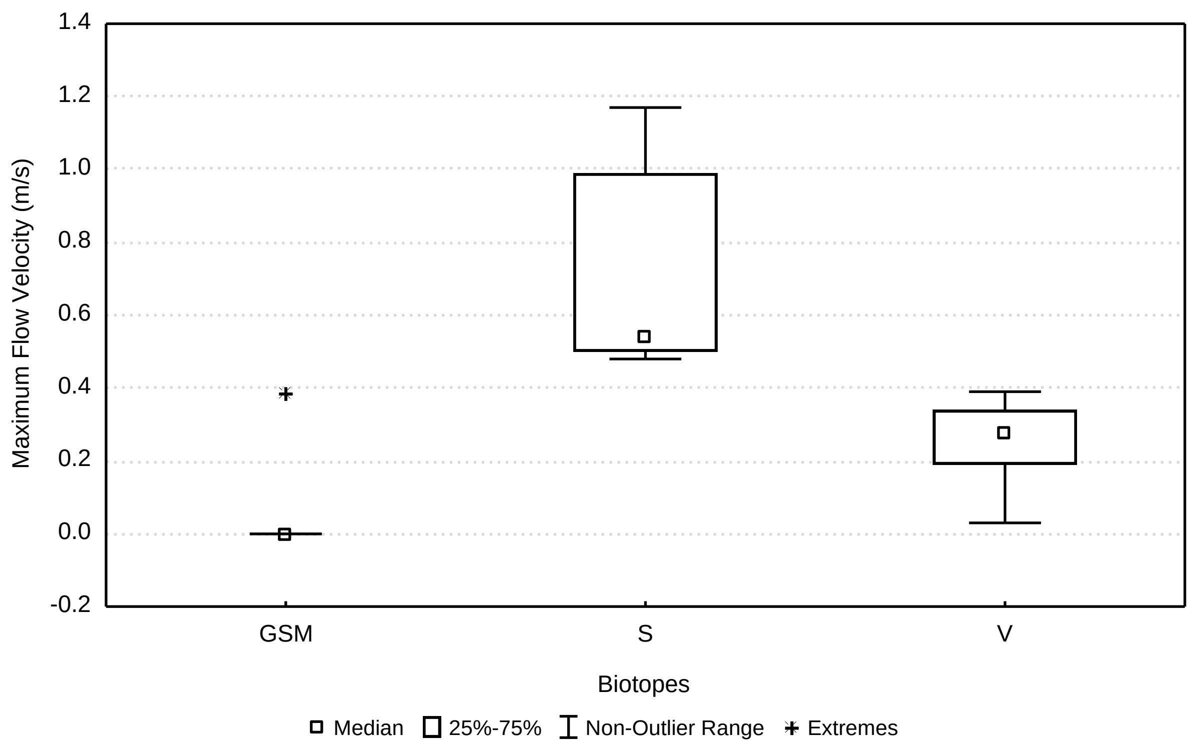

Figure 4.5: The MFV (Maximum flow velocity) of the FLOW phases (2006, 2008 and 2010) grouped into the three different biotopes: Gravel, Sand and Mud (GSM), Stones (S) and Vegetation (V).

The Vegetation biotope experienced a minimum MFV of 0.03m/s and maximum MFV of 0.39m/s (the maximum being equal to the extreme point of the GSM biotope). This was significantly lower than the MFV experienced by the Stones biotope (minimum: 0.48m/s; maximum: 1.17m/s).

Summary:

According to the data collected during the FLOW phase at sites EWR 3 and EWR 4 in the Seekoei River:

1. The GSM biotope typically experienced no flow or extremely low flow (Median: 0m/s). If flow is present the MFV can be higher than or equal to that of the Vegetation biotope, but lower than the MFV in the Stones biotope;
2. The maximum MFV measured in the Vegetation biotope (Median: 0.28m/s) is lower than in the Stones biotope; and
3. The highest flow rate at a site occurs in the Stones biotope (Median: 0.54m/s).

The flow experienced at a site can be extremely variable and depends on various factors, such as the structural features of the specific site. The flow will therefore vary from one river to another and, more importantly, could also be biotope or site specific.

CHAPTER 5
RESULTS AND DISCUSSIONS SECTION 2: MACROINVERTEBRATES OF THE SEEKOEI RIVER

5.1. OVERVIEW OF BAETIDAE AND SIMULIIDAE IN THE SEEKOEI RIVER

The study was conducted on two macroinvertebrate families present in the Seekoei River: Baetidae and Simuliidae. These families are represented by a total of 5959 individuals of which 2.8% (168) could not be identified to species level because the specimens were either too small to identify or broken. These specimens were, however, identified to family level and included in the total number of Simuliidae/Baetidae individuals. The 97.2% identified specimens are represented by a total of 11 species, as listed in Table 5.1.

Table 5.1: A species list for all the Baetidae and Simuliidae species that were found in the Seekoei River for the duration of the current study.

FAMILY	SPECIES/GENUS	FAMILY	SPECIES/GENUS
Simuliidae	*Simulium adersi*	**Baetidae**	*Cloeon* sp.
	Simulium damnosum		*Pseudocloeon vinosum*
	Simulium hargreavesi		*Pseudocloeon* sp. A
	Simulium gariepense		*Nigrobaetis* sp.
	Simulium nigritarse		*Baetis harrisoni*
	Simulium ruficorne		

Baetidae Nymphs:

Baetidae nymphs in the Seekoei River were represented by five species in the current study, i.e. *B. harrisoni*, *Cloeon* sp., *Nigrobaetis* sp., *Pseudocloeon* sp. A and *P. vinosum* (Table 5.1). For this study the Baetidae specimens from the 2006 and 2007 samples were identified, thus covering one FLOW phase and one POOLS phase. Baetidae consisted of a total number of 1030 individuals, of which almost 3.5% (36) could not be identified to species level. The abundance for each Baetidae species present in the Seekoei River for the above mentioned duration is provided in Figure 5.1. These abundances are as follows (in descending order): *Cloeon* sp. = 866, *P. vinosum* = 58, *Pseudocloeon* sp. A = 30, *Nigrobaetis* sp. = 29 and *B. harrisoni* = 11.

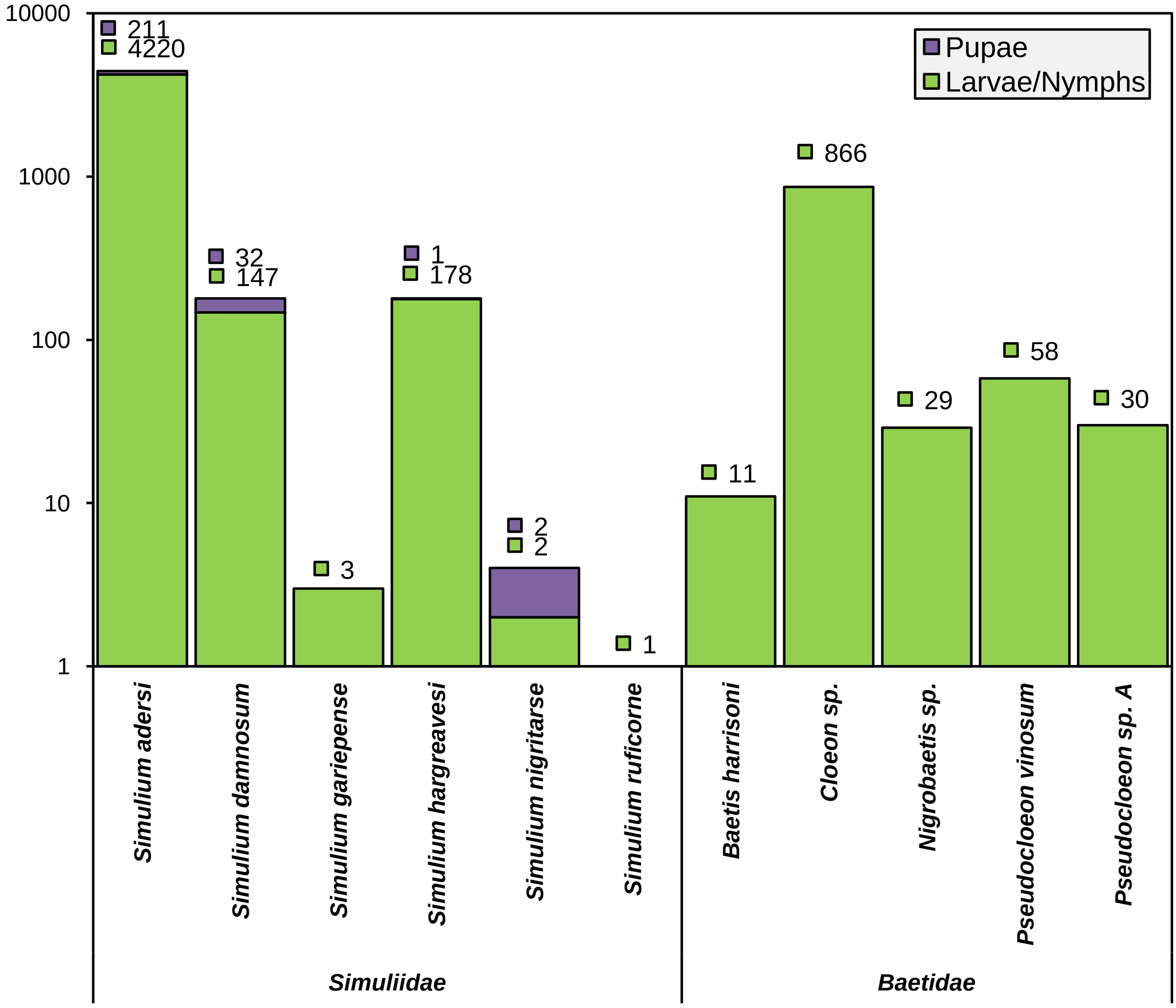

Figure 5.1: The total number of individuals for the Simuliidae (2006 – 2010) and Baetidae (2006 – 2007) species identified at site EWR 3 and EWR 4 in the Seekoei River. Simuliidae larvae and pupae are indicated separately. The y-axis has a logarithmic scale (base 10). The abundance of each species (Simuliidae larvae and pupae separately) is indicated at the top of each bar.

Simuliidae Larvae and Pupae:

Simuliidae consist of larvae and pupae, and is represented by six species in the Seekoei River during the current study, i.e. *S. adersi*, *S. damnosum*, *S. gariepense*, *S. hargreavesi*, *S. nigritarse* and *S. ruficorne* (Table 5.1). Simuliidae specimens from 2006 – 2010 were identified for this study, thus including five years and all identified hydrological phases as discussed in Chapter 4. The family Simuliidae consists of a total number of 4929 individuals, of which 2.7% (132) could not be identified to species level. The total number of individuals present in the Seekoei River for the five year period for each Simuliidae species is provided separately for the larvae and pupae in Figure 5.1. The overall abundances for each species, in descending order, are: *S. adersi* (4431), *S. damnosum* (179), *S. hargreavesi* (179), *S. nigritarse* (4), *S. gariepense* (3) and *S. ruficorne* (1).

Approximately 5% (246) of the Simuliidae individuals were pupae, represented by only four of the six species, i.e. *S. adersi*, *S. damnosum*, *S. hargreavesi* and *S. nigritarse* (Figure 5.1). Simuliidae pupae were recorded only during 2006 (only site EWR 4) and 2010 (site EWR 3 and EWR 4). *S. adersi* was the most abundant with 211 (85.77%) pupae. The *S. adersi* pupae (if present at all) were found in the Stones biotope and occasionally also in the other biotopes, making this species the only pupae that occurred in all three biotopes. *S. adersi* pupae were found in the Vegetation biotope only at site EWR 4 and in the GSM biotope only during 2010 at site EWR 4. During 2010 site EWR 4 experienced moderate MFV in the GSM biotope therefore enabling *S. adersi* pupae to be present also in the GSM biotope. This resulted in *S. adersi* pupae being more abundant in the Stones biotope and less abundant in the GSM biotope.

S. damnosum, *S. hargreavesi* and *S. nigritarse* pupae were found only in the Stones biotope. S. *damnosum* pupae was only found at site EWR 4, while *S. hargreavesi* and *S. nigritarse* pupae were found only at site EWR 3.

Summary:

During the current study *Cloeon* sp. was the most abundant Baetidae species in the Seekoei River, comprising roughly 87% of all the Baetidae individuals. The reason for its high numbers could be because they are very well adapted to no-flow/lentic systems, and prefer the pools and slow-flow areas in rivers (Agnew, 2008; Barber-James & Lugo-Ortiz, 2003). During the two years for the samples identified, the GSM and Vegetation biotopes experienced only slow or very slow MFV rates and these are the only biotopes where *Cloeon* sp. was found. It would, however, be interesting to study *Cloeon* sp. presence during the other three samples not included in this study, since some of the Vegetation biotope samples experienced moderate flow rates.

B. harrisoni was expected to be abundant, because it is a common South African mayfly known to be tolerant of polluted water as well as various flow ranges (0.1 – 1.0 m/s) (Pereira-da-Conceicoa *et al.*, 2012). Instead, *B. harrisoni* was the least abundant species of Baetidae and present only during the two 2006 samples. The recorded MFV during the 2006 and 2007 site visits (March) was relatively low or had no flow. This, however, could not have been the reason for their low numbers, because Uys (1997) also found *B. harrisoni* in the POOLS and NO-FLOW conditions. At this stage the only explanation for their low abundance is that *B. harrisoni* was possibly outcompeted by *Pseudocloeon* sp. (which occur in various flow conditions – Table 2.3), because according to Barber-James & Lugo-Ortiz (2003) both species generally occur under stones.

The most abundant Simuliidae species in the Seekoei River was *S. adersi* comprising nearly 90% of the Simuliidae (taking into account both the larvae and pupae). *S. adersi* is a very tolerant species, adapted to a wide range of conditions, and can easily reach high abundances when given the opportunity (Craig & Mary-Sasal, 2013; de Moor, 2003; Palmer & de Moor, 1998). High abundances of *S. adersi* can result from pollution or a disturbance, because they react fast to change (Palmer & de Moor, 1998). The Seekoei River is known to have no major pollution, therefore the high *S. adersi* numbers are possibly the result of a disturbance. This disturbance was probably the frequent no-flow conditions, thus when flow starts again the slower responding species gives the fast responding species, including *S. adersi*, an opportunity to increase drastically.

The least abundant species of Simuliidae was *S. ruficorne* with only one larva identified at site EWR 3 in March 2010. This low abundance in the Seekoei River should be seen as an unexpected finding, because *S. ruficorne* is known to be common in non-perennial rivers and are also tolerant to a range of conditions (Palmer & de Moor, 1998). Although *S. ruficorne* have also been found in moderate to fast currents, they prefer slow to very slow flow (Palmer & de Moor, 1998; Fain & Elsen, 1973). Thus, *S. ruficorne* probably colonised this reach/river for the first time (found only in March 2010, EWR 3 sample) and occurred in very low numbers because of the moderate flow rate. *S. ruficorne* is naturally present in low-flow environments – less than 0.1m/s (Louw *et al.*, 2013); therefore one could also argue that this species occurred in low abundances because of the moderate flow rate experienced during time of sampling.

In the Seekoei River Simuliidae pupae tend to prefer the Stones biotope because all four species identified as pupae occurred in the Stones biotope of which three were found only in the Stones biotope (i.e. *S. damnosum*, *S. hargreavesi* and *S. nigritarse*). The other species, *S. adersi*, occurred more frequently and in higher abundances in the Stones biotope. A study in the Vaal River by de Moor *et al.*, (1986) found that *S. adersi* pupae were limited to the partially submerged stones in the rapid areas. This could most likely be the reason for all the Simuliidae, not only *S. adersi*, pupae's preference for the Stones biotope.

The larvae and pupae are discussed separately only in this section, from here onwards the results will consist of the larvae and pupae combined.

5.2. BAETIDAE AND SIMULIIDAE FAMILY ABUNDANCES IN THE SEEKOEI RIVER

The total Baetidae individuals (also separated into three biotopes: GSM, Stones and Vegetation) per site visit (EWR 3 and EWR 4 as separate samples) were used during abundance analysis at family level. Figure 5.2 visually illustrates the variance between the FLOW phase and POOLS phase, in terms of the Baetidae abundances.

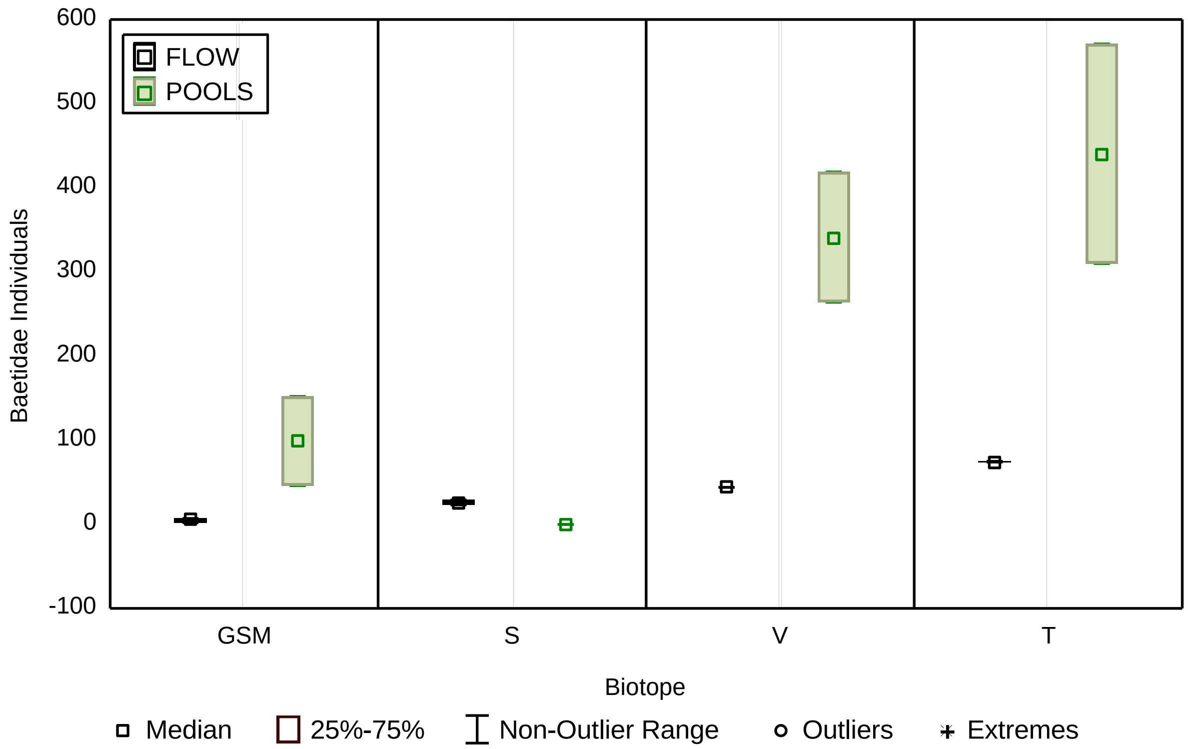

Figure 5.2: The abundance of the family Baetidae in the Seekoei River (2006-2007), presenting the differences between the FLOW phase and the POOLS phase. The data are grouped as following: GSM (Gravel, Sand and Mud), S (Stones), V (Vegetation) and T (Total Baetidae individuals recorded per site visit, thus not separated into biotopes).

From Figure 5.2 it is clear that the total Baetidae individuals recorded per site visit (T) was significantly higher during the POOLS phase (Median = 440.5) than the FLOW phase (Median = 74.5). In both hydrological phases (FLOW and POOLS) the Vegetation biotope had the highest abundance of Baetidae individuals.

The Baetidae abundance in the GSM biotope was significantly higher during the POOLS phase compared to the FLOW phase, with the Medians being 99 and 4.5 respectively. The same is true in the Vegetation biotope with a Median of 341.5 (higher) for the POOLS phase and 44 for the FLOW phase. The abundance in the Stones biotope, however, was significantly lower during the POOLS phase (Median = 0) than the FLOW phase (Median = 26), because the Stones biotope was not sampled during the POOLS phases.

In the Seekoei River Simuliidae were absent from both sites during the POOLS phase but were always present during the FLOW phase. Figure 5.3 therefore only illustrates the variation in the abundances between the three biotopes during the FLOW phase.

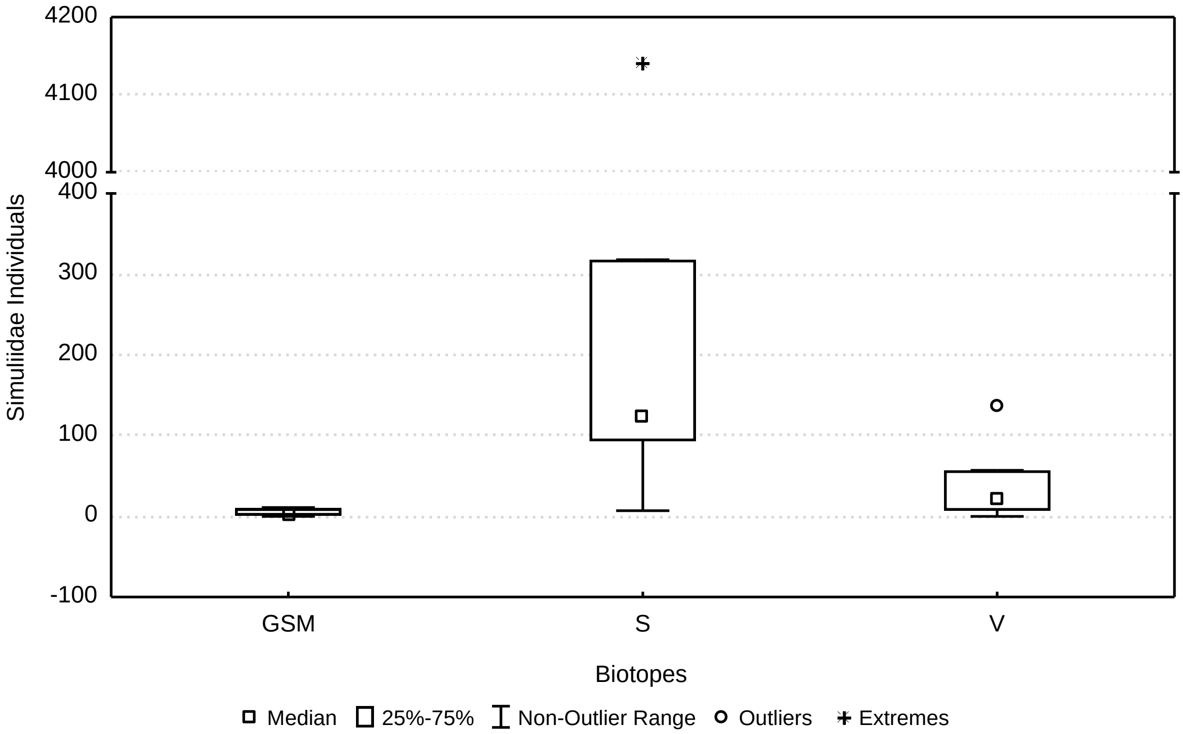

Figure 5.3: The abundances representative of the Simuliidae family during the FLOW phase (2006, 2008 and 2010) in the Seekoei River, grouped into the three biotopes: GSM (Gravel, Sand and Mud), S (Stones) and V (Vegetation).

The GSM biotope had very low numbers of Simuliidae individuals (Median = 2) and the highest numbers occurred in the Stones biotope (Median = 123). The abundance found in the Vegetation (Median = 21) biotope was between that of GSM and Stones.

<u>*Summary:*</u>

According to the current data, the Baetidae abundances are significantly different between the FLOW phase and POOLS phase. Baetidae of the Seekoei River are more abundant, therefore also more adapted, to POOLS phase conditions. This is true especially for *Cloeon* sp. which was the only species more abundant in the

POOLS. *Cloeon* sp. also contributed most to the Baetidae abundances; the reason for highest abundances recorded in the Vegetation biotope (during both the POOLS and the FLOW phases). *Cloeon* sp. was also common in the POOLS phase according to the findings of Uys (1997). Barber-James & Lugo-Ortiz (2003) commented that *Cloeon* sp. prefers the vegetation areas during slow or no-flow conditions. The abundances can also be influenced by the community structure, because when predators are few/absent or less species are present, predation or competition is reduced, giving those that are present the opportunity to flourish (Watson, 2009; Growns, 1998).

For the duration of this study Simuliidae have only been recorded in the FLOW phase, because generally species of Simuliidae rely on flow conditions for survival. Some species can however, survive in no-flow conditions, such as *S. ruficorne* (de Moor, 2003).

In the Seekoei River Simuliidae seems to prefer the Stones biotope; that was where the highest abundances occurred (Figure 5.3). The Simuliidae abundances of the Stones biotope were especially high due to the high numbers of *S. adersi*. A study by Uys (1997) indicated that Simuliidae species, especially *S. adersi*, typically occurs in the Stones-in-current, as was found to be the case during this study.

5.3. BAETIDAE AND SIMULIIDAE SPECIES COMPOSITION IN THE SEEKOEI RIVER

5.3.1. Within-site Distribution of Species: POOLS Phase and FLOW Phase

<u>*Species distribution during the POOLS phase:*</u>

During the POOLS phase all six of the Simuliidae species were absent and only two of the five Baetidae species were present, which are *Cloeon* sp. and *P. vinosum* (Table 5.2). The POOLS phase data includes two years (2007 and 2009) for the Simuliidae species and only one year (2007) for the Baetidae species.

Table 5.2 indicates that during the POOLS phase *Cloeon* sp. was absent from the Stones biotope, because no Stones biotope were sampled during the POOLS phase. *Cloeon* sp. therefore occurred only in the GSM and Vegetation biotopes at both sites. *P. vinosum* was recorded during the POOLS phase only in the GSM biotope at site EWR 3.

Table 5.2: Baetidae and Simuliidae species presence in the Seekoei River according to biotope (Gravel, Sand and Mud (GSM)/Stones/Vegetation), site (EWR 3/EWR 4) and hydrological phase (FLOW phase (F)/POOLS phase (P)).

		GSM		Stones		Vegetation	
Family	**Species/Genus**	**EWR 3**	**EWR 4**	**EWR 3**	**EWR 4**	**EWR 3**	**EWR 4**
Simuliidae	***Simulium adersi***	F	F	F	F	F	F
	Simulium damnosum			F	F	F	F
	Simulium hargreavesi			F	F	F	F
	Simulium gariepense	F				F	
	Simulium nigritarse			F			
	Simulium ruficorne			F			
Baetidae	***Cloeon* sp.**	F/P	P			F/P	F/P
	Pseudocloeon vinosum	P		F	F	F	
	***Pseudocloeon* sp. A**		F		F		F
	***Nigrobaetis* sp.**					F	F
	Baetis harrisoni				F	F	

Species distribution during the FLOW phase:

All of the 11 recorded Baetidae and Simuliidae species, which include six Simuliidae species and five Baetidae species, were present during the FLOW phase (Table 5.2). The FLOW phase data includes three years (2006, 2008 and 2010) for the Simuliidae species and only one year (2006) for the Baetidae species.

While most Simuliidae and Baetidae species occurred at both sites, some species were found at only one of the sites, thus either EWR 3 or EWR 4 (Table 5.2). *S. adersi*, *S. damnosum* and *S. hargreavesi* were found at both sites, EWR 3 and EWR 4. The other three Simuliidae species, *S. gariepense*, *S. nigritarse* and *S. ruficorne*, occurred only at site EWR 3. All the Baetidae species were found at both sites, except for *Pseudocloeon* sp. A that occurred only at site EWR 4.

S. adersi was present in all three of the biotopes, while *S. damnosum* and *S. hargreavesi* occurred only in the Stones and Vegetation biotopes. This is true according to other literature which states that *S. adersi* are present in a diversity of habitats, whereas both *S. damnosum* and *S. hargreavesi* prefer the Stones and Vegetation biotopes of the rapids (Craig & Mary-Sasal, 2013; de Moor, 2003). These two species also seem to find the habitat at site EWR 3 more favourable, because both species were more abundant at this site. The long rapid (Figure 3.3) of site EWR 3 could be one reason for their preference, because the highest abundances of *S. damnosum* and *S. hargreavesi* always occurred in the Stones biotope.

S. gariepense was recorded in the GSM and Vegetation biotopes, but *S. nigritarse* and *S. ruficorne* were recorded only in the Stones biotope. It was not expected to find *S. gariepense* in the Seekoei River because this species are known to be restricted to rivers that are large and turbid (Louw *et al.*, 2013; Palmer & de Moor, 1998). The possibility exists that the large pool (Figure 3.4) at site EWR 3 is the ideal habitat (conditions similar to large rivers due to the size of the pool) and because no earlier studies have been done in the Seekoei River in order to confirm their distribution in the Seekoei River. Both *S. nigritarse* and *S. ruficorne* can be expected to be present in the Seekoei River because they are tolerant species, known to colonise newly formed streams (de Moor, 2003; Louw *et al.*, 2013; Palmer & de Moor, 1998). Palmer & de Moor (1998) also found that these species occur on/under stones, and this study also suggests that *S. nigritarse* and *S. ruficorne* prefer the Stones biotope. Uys (1997) found *S. nigritarse* during the ONSET phase which was preceded by a POOLS phase. Therefore one can also argue that *S. nigritarse* was possibly found in low numbers during the FLOW phase, because the FLOW phase probably just started after an ONSET phase preceded by a POOLS phase. These individuals could then be the few still present, and would probably not be present for long.

During the FLOW phase *Cloeon* sp. was always present in the Vegetation biotope and at one of the sites in the GSM biotope, but never in the Stones biotope during FLOW phase. Chakona *et al*. (2008) found during a study on non-perennial rivers that low numbers of *Cloeon* sp. occurs only in the Vegetation biotope during flow conditions, while Williams (1987) commented that *Cloeon* sp. is well adapted to no-flow. *Cloeon* sp. therefore prefer the Vegetation biotope during flow conditions in the Seekoei River and at site EWR 3 this species also occurred in the GSM biotope (located in the large pool – Figure 3.4) which experienced near to no-flow conditions.

P. vinosum occurred mostly in the Stones but also the Vegetation biotopes and not the GSM biotope, while an unidentified *Pseudocloeon* species, *Pseudocloeon* sp. A, was found in all three biotopes (highest abundance in Stones biotope). When *Pseudocloeon* sp. A and *P. vinosum* are considered together, this genus are more abundant in the Stones biotope but also occur in all the biotopes. A study by Chakona *et al*. (2008) agree and also found *Pseudocloen* to be more abundant in the cobbles (stones), but it was present in all the habitat types in Zimbabwean non-perennial rivers.

A *Nigrobaetis* sp. was found only in the Vegetation biotope and *B. harrisoni* occurred in the Vegetation and Stones biotopes. According to Barber-James & Lugo-Ortiz (2003) *Nigrobaetis* sp. prefers the riffles in fast flow; therefore it was not expected to find this species only in the Vegetation biotope. *Nigrobaetis* sp. was however more abundant at EWR 4 where it is possible that most of the vegetation is found in riffle areas (less vegetation in the riffles of site EWR 3). It is therefore possible that *Nigrobaetis* sp. prefers the vegetation in riffle areas during very slow to moderate flow conditions in the Seekoei River.

B. harrisoni was expected to be abundant and only in the Stones biotope (Barber-James & Lugo-Ortiz, 2003), but this was not the case. This is probably due to the habitat structure at the sites. Watson (2009) demonstrated that during March 2006 the substrate composition mainly consists of bedrock and boulders at site EWR 4, and mainly boulders at site EWR 3 (with few cobbles). Barber-James & Lugo-Ortiz (2003) stated that *B. harrisoni* are found under small to medium stones, which was not the abundant substrate present at the time. Chakona *et al*. (2008) also found the genus, *Baetis*, to be most abundant in the cobble habitat.

Summary:

Simuliidae species were more diverse at site EWR 3 with only three of the six identified species found at EWR 4. This suggests that the habitat structure present at site EWR 3 is possibly more suitable to the preferences of *S. gariepense* (the large pool), *S. nigritarse* (the rapid) and *S. ruficorne* (the rapid), which occurred only at this site. It is, however, suggested that more research be done on this aspect because of the limited data on these species in the Seekoei River.

In the Seekoei River Simuliidae species were entirely absent from the POOLS phase while Baetidae species diversity was lower in the POOLS phase than the FLOW phase. In the previous section (Section 5.2) Baetidae abundances was however, found to be significantly higher in the POOLS phase than the FLOW phase. In other words, different to Simuliidae, Baetidae occurs in higher abundances during the POOLS phase, but is more diverse in the FLOW phase.

The higher abundances of Baetidae, in POOLS phase compared to the FLOW phase, could not be confirmed in literature. Studies comparing near similar conditions seem to rather find the opposite or that both diversity and abundance is higher in the riffles, which have similar conditions to the FLOW phase in this study (Brown & Brussock, 1991; Logan & Brooker, 1983). According to Brown & Brussock (1991) the findings of various studies differed in terms of the macroinvertebrate diversity and density (abundance) when comparing the pools with the riffles. These differences seem to be mainly due to different river types and especially the habitat structure, e.g. mountain streams, gravel-bed streams, or lowland sand-bed streams. Minshall & Minshall (1977) found that Chironomidae abundances, for instance, was higher in the pool areas and concluded that it could be because of the available substrate. When pools experience less severe disturbances caused by flow variability, the remaining substrate is more suitable to provide refuge for macroinvertebrates (Brown & Brussock, 1991). This suggest that the pools at sites EWR 3 and EWR 4 possibly provide sufficient substrates thus, also sufficient refuge for the few Baetidae species present, therefore Baetidae are able to occur in high abundances. This demonstrates just how important these pools can be as refugia to certain species.

5.3.2. Flow Preferences of Simuliidae Species in the Seekoei River

In order to determine if any of the Simuliidae species have a flow preference in the Seekoei River a Cluster analysis was used. To eliminate the FLOW phase samples with no Simuliidae species only the FLOW phase samples with at least one species present were selected as data. The Bray Curtis Similarity Cluster analysis between the samples identified three distinct groups at 60% similarity as indicated by Figure 5.4. The MFV recorded for each sample is used to determine the flow rate range for each group. These Groups with their MFV ranges are as following:

Group 1: All samples including *S. gariepense*. MFV range = 0m/s – 0.19m/s.
Group 2: All samples including only *S. adersi*. MFV range = 0m/s – 0.5m/s.
Group 3: All samples including one or more of the following species *S. damnosum*, *S. hargreavesi*, *S. nigritarse* and *S. ruficorne*. MFV range = 0.34m/s – 1.17m/s.

The MFV range of Group 2 overlap with both Group 1 and Group 3 and *S. adersi* (the only species found in Group 2) were found in all three groups. The MFV range of Group 1 and Group 3 differs significantly from each other, as Group 3 has a higher MFV range than that of Group 1. In other words, while *S. adersi* was found at all three MFV ranges, the other four species from Group 3 (*S. damnosum*, *S. hargreavesi*, *S. nigritarse* and *S. ruficorne*) were found only during higher flow rates (MFV range = 0.34m/s – 1.17m/s) and *S. gariepense* (Group 1) was found only at lower flow rates (MFV range = 0m/s – 0.19m/s).

The MDS ordination of the same data (used for Cluster analysis) yielded a good (stress = 0) two dimensional representation of the sample clusters (Figure 5.5). The three groups identified by the Cluster analysis were confirmed by the MDS ordination.

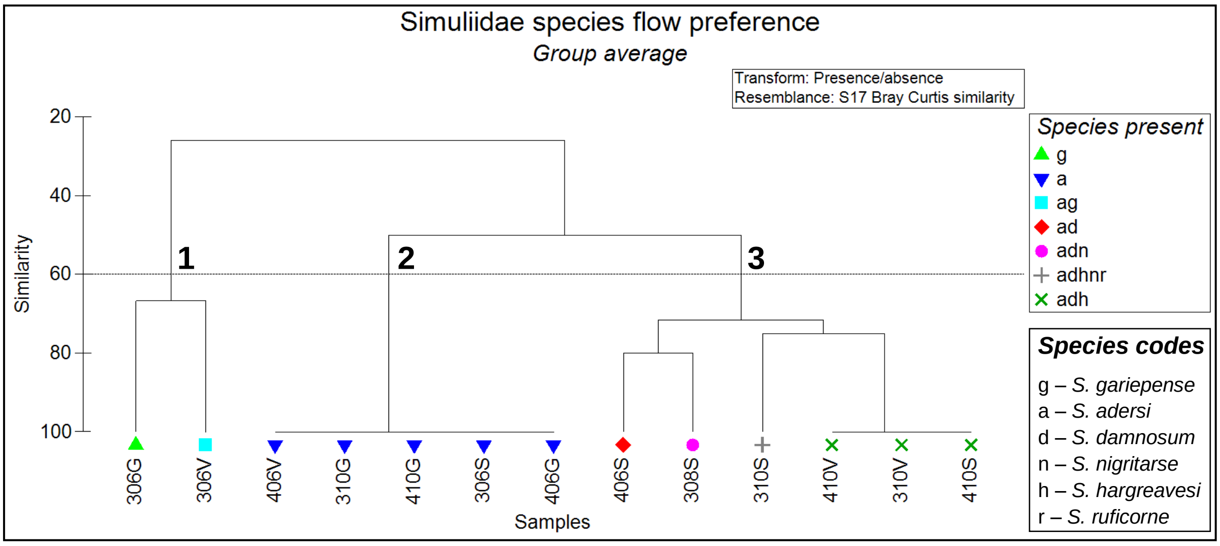

Figure 5.4: Cluster analysis of Simuliidae species present in each biotope at sites EWR3 and EWR4 in the Seekoei River from March 2006 to October 2010. Samples where at least one Simuliidae species were present were included in the analysis.

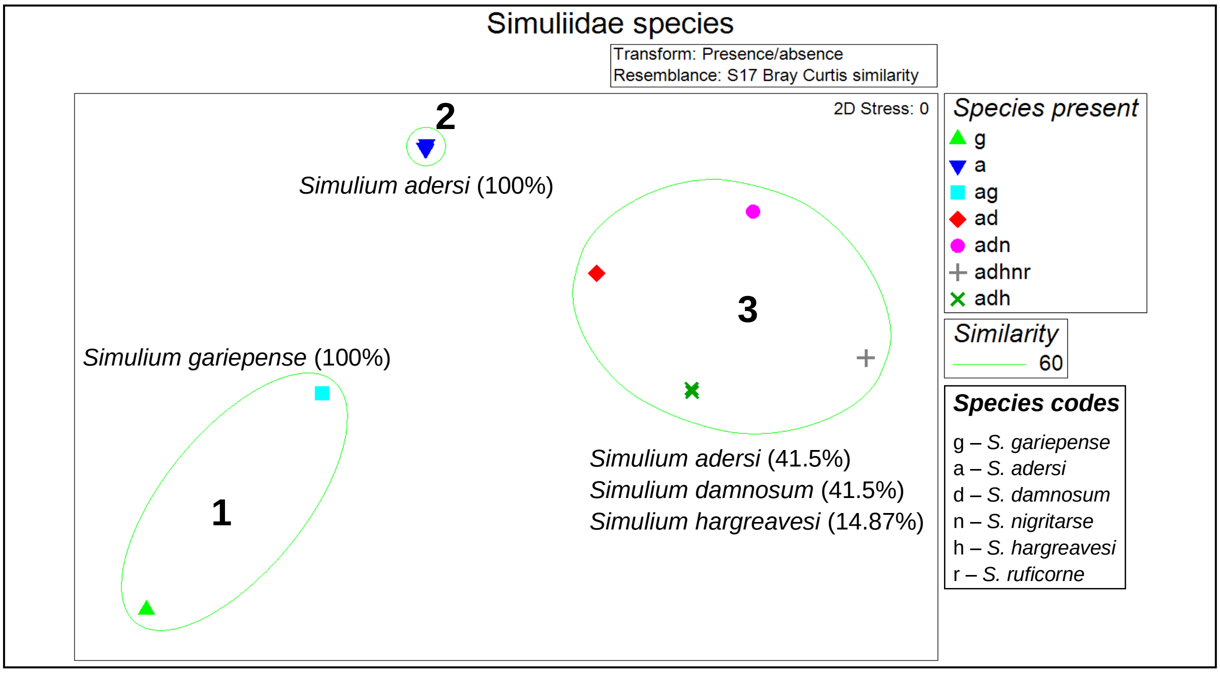

Figure 5.5: MDS ordination of Simuliidae species present in each biotope at sites EWR3 and EWR4 in the Seekoei River from March 2006 to October 2010. Selected only the samples where at least one Simuliidae species were present. Indicates which species contributed most to the similarity (determined by a SIMPER test) within each of the groups.

The resemblance matrix of the same presence/absence data (used for Cluster analysis) was used to do an ANOSIM test (for full results of the ANOSIM test see Appendix C: Table C1). The following null hypothesis was tested:

"There is no difference between the Groups"

The sample statistic resulted in a Global R = 0.956 and a significance level = 0.1% (which in PRIMER format is equal to $p < 0.001\%$). These results indicate that the null hypothesis can be rejected, because Global R is higher than 0. The very low significance level ($p < 0.001$) indicates that there is a highly significant difference between the Groups in terms of the species composition in each sample.

Even though all groups differ significantly from each other, the results of the pairwise test suggest that Group 1 and Group 3 is the most different from each other. It should however be noted that the number of permutations was very low, but the difference is significant (significance level is less than 5% or $p < 0.05$).

The same presence/absence data (used for Cluster analysis) was used to do a SIMPER test (for full results of the SIMPER test see Appendix C: Table C2). The SIMPER test determined which species contributed most to the similarity within a group (Figure 5.5) as well as which species contributed most to the dissimilarity between the groups.

The samples from Group 2 were the most similar with an average similarity of 100% and *S. adersi* contributed to all of the similarity within the group. Group 3 showed an average similarity of 78.48% and the highest contributing species were *S. adersi* (41.5%), *S. damnosum* (41.5%) and *S. hargreavesi* (14.87%). Group 1 had the lowest average similarity (66.67%) with S. *gariepense* contributing 100% to the similarity within the group.

The results further indicate that Groups 2 & 3 and Groups 1 & 2 differs less from each other (average dissimilarity (AD) of 50% and 66.67% respectively) than the dissimilarity experienced between Groups 1 & 3 (AD of 80.12%). The highest contributing species responsible for the dissimilarity between the groups are as follows:

<u>Groups 1 & 3</u> (AD = 80.12%)
Simulium damnosum (28.01%)
Simulium gariepense (28.01%)
Simulium hargreavesi (17.26%)
Simulium adersi (15.6%)
Simulium nigritarse (7.9%)

<u>Groups 1 & 2</u> (AD = 66.67%)
Simulium gariepense (62.5%)
Simulium adersi (37.5%)

<u>Groups 2 & 3</u> (AD = 50%)
Simulium damnosum (50%)
Simulium hargreavesi (30.56%)
Simulium nigritarse (13.89%)

The dissimilarity between Group 1 and Group 3 (which differs the most) was mostly contributed by *S. damnosum* (28.01%) from Group 3 and *S. gariepense* (28.01%) from Group 1.

Combining all of the results of this section it is suggested that, because of the significant difference between Groups 1 and 3 and the species contributing most to the similarity within these groups, the species contributing most to the differences possibly prefer the allocated MFV range. Therefore *S. damnosum* and maybe also *S. hargreavesi* seems to prefer higher MFV ranges (0.34m/s – 1.17m/s), while *S. gariepense* seems to prefer lower MFV ranges (0m/s – 0.19m/s) in the Seekoei River. The other species, except *S. adersi*, was present only in the higher MFV ranges, but had a low frequency (present only in one or two samples). *S. adersi* was found in all three Groups; therefore it seems that *S. adersi* does not have a flow preference in the Seekoei River and is found in a wide MFV range (0m/s – 1.17m/s).

Summary:

Some of the Simuliidae species (not all) do seem to have certain flow preferences in the Seekoei River. Although it can be very challenging to determine the flow ranges of specific species 100% accurately, it is possible to give a rough estimation in terms of the MFV. This study clearly indicates that even though the family was present at any of the flow ranges in the Seekoei River, some species are absent during certain flow ranges, i.e. species diversity change with different flow rates. Rivers-Moore *et al.* (2006) also found different flow preferences for certain Simuliidae species in other South African rivers. They found two species preferring low flow rates (*S. rutherfoordi* and *S. impukane* = 0.3m/s) and two with a higher flow rate preference (*S. merops* = 0.7m/s; *S. nigritarse* = 0.8m/s to 0.9m/s).

As expected *S. gariepense* favours lower flow, ranging from very slow flow to slow flow, in the Seekoei River because this species is adapted to slow flow conditions (de Moore, 2003). In the Seekoei River *S. damnosum* was present only in the higher flow ranges ranging from moderate flow to fast flow. De Moor (2003) commented that *S. damnosum* are usually found in fast flow, but Palmer & de Moor (1998) found high abundances of *S. damnosum* in slow- to moderate flow. *S. hargreavesi* also seems to prefer the higher flow rates of the Seekoei River because this species was present only in moderate to fast flow, but had a low frequency of occurrence. Although this species are found in various flow ranges (de Moore, 2003; Palmer & de Moor, 1998), Roberts & Okafor (1987) found that *S. hargreavesi* prefer fast flow conditions.

S. adersi have already been confirmed as a very tolerant species (Craig & Mary-Sasal, 2013), therefore it was expected to find *S. adersi* in all the flow types experienced during FLOW phase. In other words, *S. adersi* does not seem to have any flow preferences in the Seekoei River, because they were present in flow ranging from very slow to fast and were the most abundant Simuliidae species each time.

5.3.3. Summarising the Species Distribution of the Seekoei River

The following summary of all the species which occurred in the Seekoei River during the current study clearly demonstrates that the habitat and flow of a site are important environmental factors which determine the species composition of a non-perennial river. Keep in mind that during this study all the Simuliidae species were absent during the POOLS phase, thus all have a preference for flow conditions.

a) Simulium adersi

S. adersi is the Simuliidae species that is the best adapted to the flow conditions of the Seekoei River according to the results of this study. The reason is because during the FLOW phase this species was the most abundant Simuliidae species, present in all the biotopes and found during any flow range in this river. *S. adersi* does however have a preference for the Stones biotope in the Seekoei River.

b) Simulium damnosum

S. damnosum is well adapted to the flow conditions of the Seekoei River due to various reasons. Firstly, *S. damnosum* was found in moderate abundances, overall. Secondly, this species prefers the Stones and Vegetation biotopes, but seems to favour the long rapid of site EWR 3 even more (higher abundances occurred in this section). Lastly, *S. damnosum* was demonstrated to have a preference for moderate to fast flow conditions in the Seekoei River.

c) Simulium hargreavesi

Although *S. hargreavesi* does not normally occur in non-perennial rivers (Palmer & de Moor, 1998), this species was present in the Seekoei River (an ephemeral river) and seems to be well adapted to its flow conditions. One of the reasons is that *S. hargreavesi* was present in moderate abundances. This species also prefers the Stones and Vegetation biotopes (like *S. damnosum*), and also seems to favour the long rapid of site EWR 3 even more (higher abundances occurred in this section). *S. hargreavesi* also seems to have a preference for moderate to fast flow conditions in the Seekoei River.

d) Simulium gariepense

S. gariepense should be considered as an important species, because it is endemic to the Orange River Basin (Louw *et al.*, 2013). This species is known as limited to large rivers, therefore its presence in the Seekoei River could be considered as a new distribution. It is therefore possible for *S. gariepense* to be present in medium-sized rivers and non-perennial rivers. *S. gariepense* prefers lower flow ranges (very slow to slow flow) as well as the GSM and Vegetation biotopes (probably in the large pool) of the Seekoei River. This species occurred only during 2006 and not during the other two years that experienced a FLOW phase, therefore *S. gariepense* should be considered as an important species for conservation in South African rivers.

e) Simulium nigritarse

S. nigritarse does not seem to be well adapted to the flow conditions and habitat of the Seekoei River. This is due to the fact that this species occurred in very low abundances. *S. nigritarse* does however appear to have a preference for the rapid at site EWR 3, because it was found only in the Stones biotope at site EWR 3.

f) Simulium ruficorne

S. ruficorne appears to be the least adapted species of all the identified Simuliidae species in the Seekoei River, even though this is a tolerant species which is known to be common in non-perennial rivers. As with *S. nigritarse*, *S. ruficorne* also appears to have a preference for the rapid at site EWR 3, because it was found only in the Stones biotope at site EWR 3.

g) Cloeon sp.

Cloeon sp. is clearly more adapted to the no-flow conditions of the POOLS phase and generally prefers the Vegetation biotope in the Seekoei River. That is because firstly *Cloeon* sp. were more abundant during the POOLS phase and during both hydrological phases the highest abundances occurred in the Vegetation biotope. Secondly in the FLOW phase *Cloeon* sp. preferred the Vegetation biotope, probably because they are protected from high flow in the vegetation. Of all the identified Baetidae species in the Seekoei River *Cloeon* sp. seems to be the best adapted to non-perennial conditions. That is because *Cloeon* sp. was overall the most abundant Baetidae species.

h) Pseudocloen sp.

Two species, *Pseudocloeon* sp. A and *P. vinosum*, were identified during this study. The genus usually prefers the Stones biotope, but *Pseudocloeon* sp. A seems to prefer the Vegetation biotope. In the Seekoei River *Pseudocloeon* sp. seems to be the best adapted of all the Baetidae species to the FLOW phase, because this genus was the most abundant in the FLOW phase (of all the Baetidae species).

i) Nigrobaetis sp.

In the Seekoei River *Nigrobaetis* sp. seems to prefer the vegetation in riffle areas during very slow to moderate flow conditions.

j) Baetis harrisoni

B. harrisoni is not very abundant in the Seekoei River, because the habitat consisted of a low percentage of cobbles and other small to medium stones, which is the preferred habitat of this species. In the Seekoei River *B. harrisoni* also seems to prefer the vegetation in the riffles instead of the stones in the riffles.

5.4. SIMULIIDAE AND BAETIDAE SPECIES COMBINED AND SASS 5

To find the relationship between the NoT (the families recorded on the SASS sheet) and the SASS Score recorded according to the SASS 5 method, a correlation between the NoT and the SASS Score were computed (Figure 5.6). This was done using only the data from the March samples in 2006 to 2010 at site EWR 3 and EWR 4 separately; also take note that the data points consists of the actual SASS 5 Score and NoT as well as the scores calculated separately for each of the three biotopes. As illustrated, a significant positive correlation was found ($p = 0.0000$; $r = 0.9899$; $r^2 = 0.9798$), therefore a higher NoT generally results in a higher SASS Score. This was also found during a study with a much larger data set including various ephemeral rivers (Watson & Dallas, 2013).

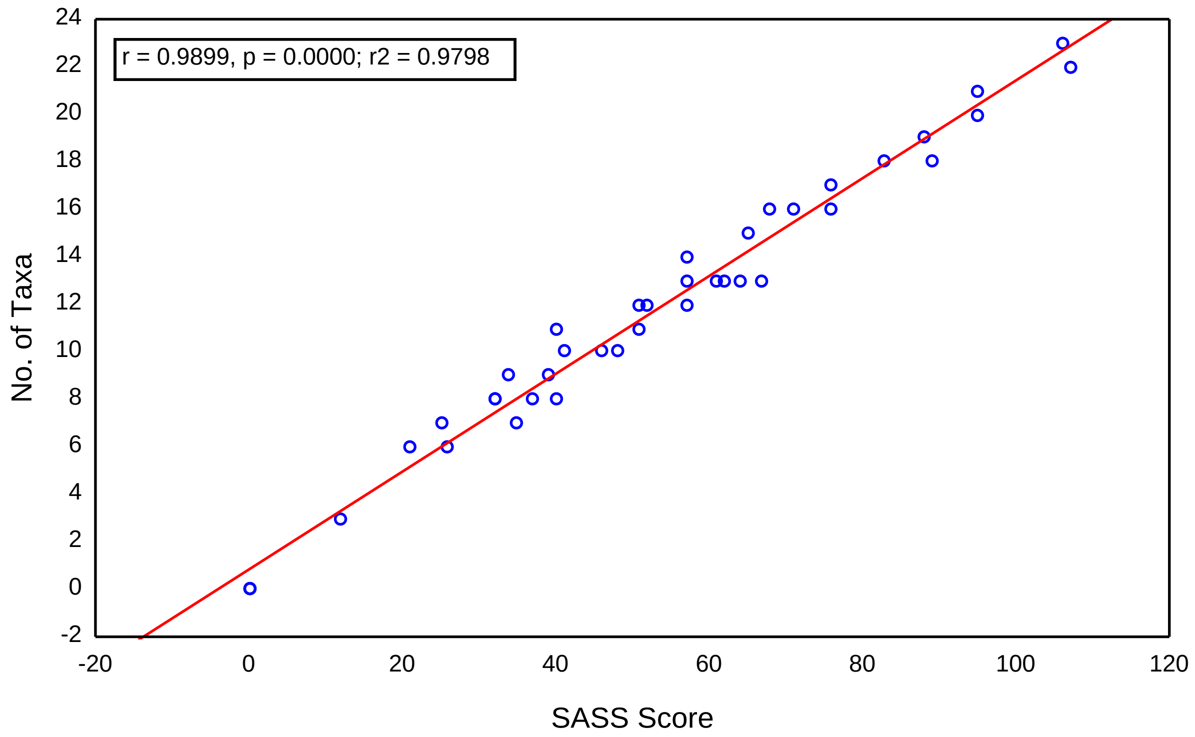

Figure 5.6: Correlation between the Number of Taxa (NoT) and the SASS Score as recorded by the SASS 5 method. The data points include the totals per site as well as the three biotopes separately for the March/April site visits during 2006 – 2010 at sites EWR 3 and EWR 4.

To understand why this strong correlation exists between the NoT and SASS Score, all the taxa (families on the SASS scoring sheet) that occurred in the Seekoei River in the samples mentioned above, were identified. Using the sensitivity scores (as allocated on the SASS 5 scoring sheet) of these families, the following is obvious in the Seekoei River:

- The maximum sensitivity score was 12, but 90% of the taxa that could be present in the Seekoei River have a sensitivity score between 1 and 8.
- The minimum sensitivity score was 1.
- Both the median and mode sensitivity score was 5.
- The percentage of taxa that could be present in the Seekoei River, with a sensitivity score of ≤5 was 65%.

In the Seekoei River, taxa with low sensitivity scores are very common (median and mode sensitivity score was 5). The families responsible are: Gerridae, Veliidae, Dytiscidae, Gyrinidae, Haliplidae, Hydrophilidae, Ceratopogonidae, Simuliidae, Tabanidae and Tipulidae. On the other hand, taxa with a sensitivity scores of 12 are only Baetidae >2 sp. and Hydropsychidae >2 sp. This also suggests that the ephemeral river macroinvertebrate community is naturally rich in hardy, adaptable generalist families with low sensitivity scores, as mentioned by Watson & Dallas (2013).

During the POOLS phase one would expect the SASS Score to be lower compared to the FLOW phase. Figure 5.7 however, illustrates that in terms of the SASS Score, no significant difference exists between the POOLS phase and the FLOW phase in the Seekoei River. This means that (because of the strong positive correlation identified above) the NoT, thus families, also do not differ significantly between the POOLS phase and FLOW phase. The SASS Score of the different biotopes also does not seem to differ considerably as proven by Dallas (2007a). Dallas (2007a) also found that the SASS Score in the Stones biotope is generally the highest of the three biotopes, but this study on the Seekoei River mostly found a higher SASS Score in the Vegetation biotopes. This is most likely due to the regular disturbances experienced within the Stones biotope (i.e. dry periods between the flow periods), which leads to the less sensitive (hardy) species surviving in the Stones biotope of the Seekoei River.

The total number of species (Baetidae and Simuliidae combined) from the March 2006 and 2007 samples (EWR 3 and EWR 4 separately) was used for the graph in Figure 5.8, because only these samples of Baetidae were identified. Figure 5.8 clearly demonstrates that there is a significant difference between the number of species present during POOLS phase (lower; median = 1.5) and the number of species during FLOW phase (higher; median = 6.5). This is mostly caused by the significant differences found between the two phases in the Stones and Vegetation biotopes. Even though this is based on only two families, Baetidae and Simuliidae, it still indicates that a significantly lower number of species occurs in the Seekoei River during the POOLS phase than is found during FLOW phase (which is not the case with family-level data as illustrated in the previous section and Figure 5.7).

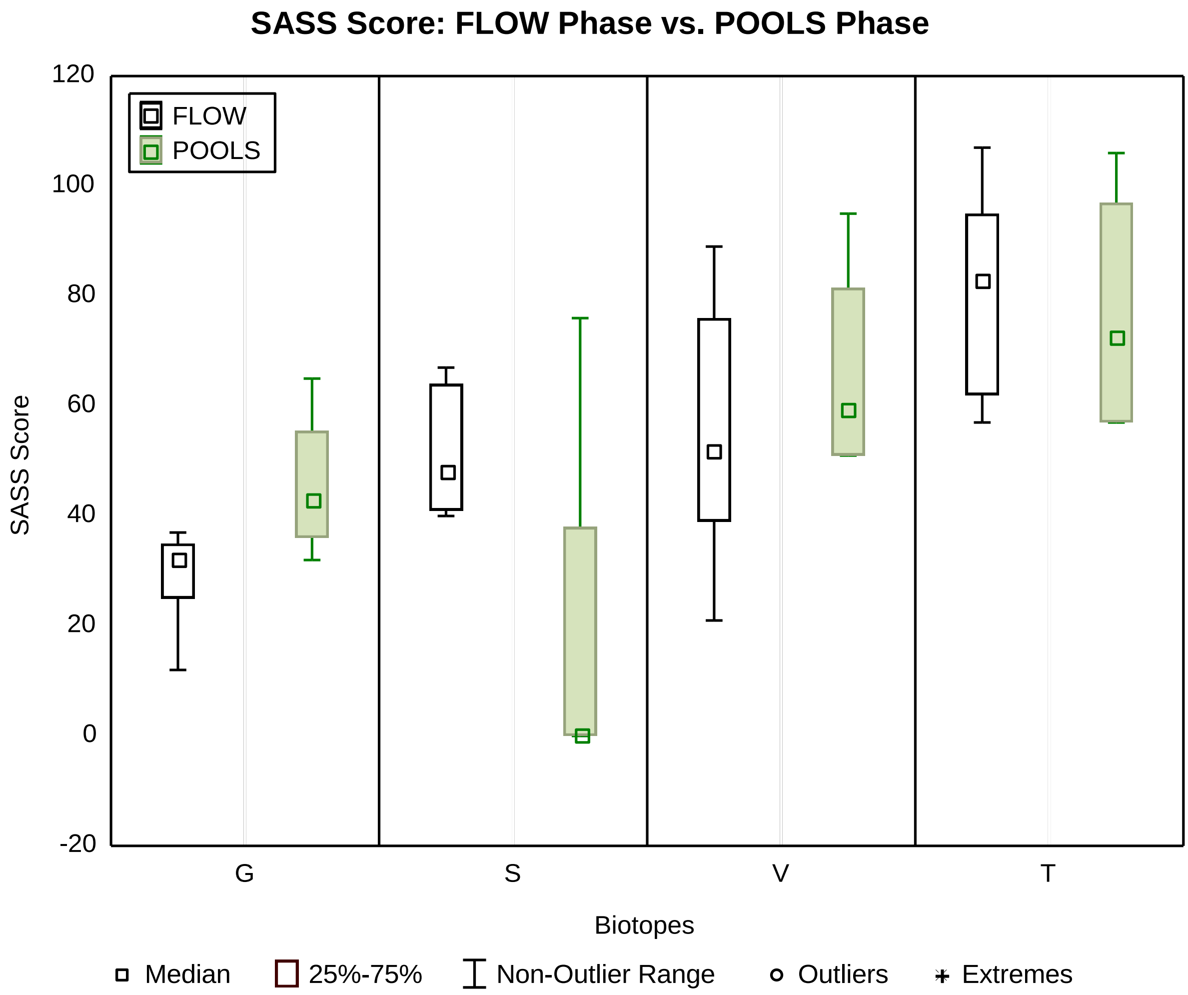

Figure 5.7: The variability of the SASS Score during the FLOW phase and the POOLS phase. The data points include the totals per site as well as the three biotopes separately for the March/April site visits during 2006 – 2010 at sites EWR 3 and EWR 4.

The majority of taxa that are found in the Seekoei River have a sensitivity score lower or equal to 5. This is true during the FLOW phase and the POOLS phase. The SASS Score is therefore generally very low, and the ASPT unreliable (Dallas, 2007b). This is because the NoT are generally low (not more than 23 taxa present during this study) in the ephemeral Seekoei River, and taxa with a sensitivity score above 5 are very few and even less with a sensitivity score above 8. In other words, the SASS Score in the Seekoei River can be relatively high because of a large number of families, all with a low sensitivity score, or because of one (or a few) taxa with a high sensitivity score among a low number of families.

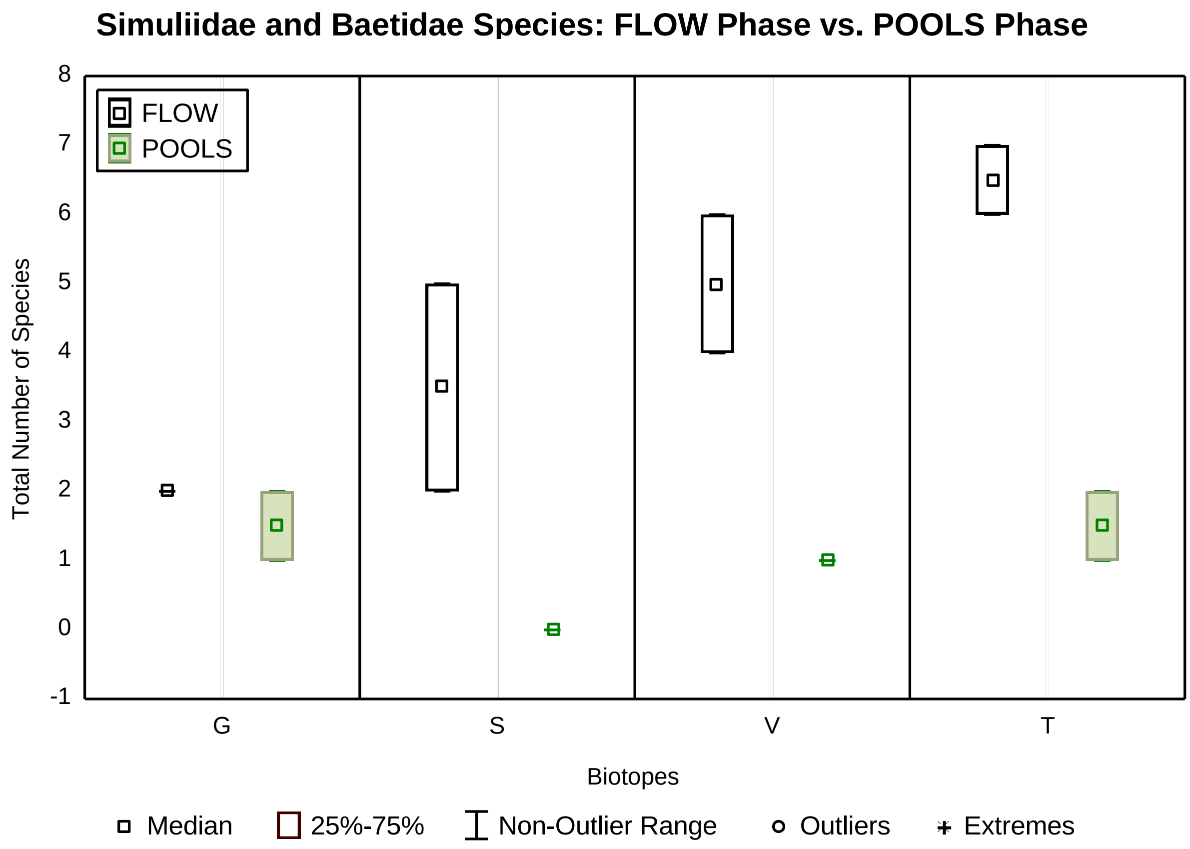

Figure 5.8: The variability of the Total number of species during the FLOW phase and the POOLS phase. The data points include the three biotopes separately for the March/April site visits during 2006 – 2007 at sites EWR 3 and EWR 4.

<u>*Implications for the interpretation of the SASS 5 biomonitoring data:*</u>

After studying Table 5.2 it was found that in some instances, at a site, in a specific biotope, only one species of a specific family could have been present in the FLOW or POOLS phase throughout the study. For Simuliidae it was first thought that this occurred only at site EWR 4 in the GSM biotope, but when the raw data was studied in detail this phenomenon was found often in the GSM biotope at both sites and during 2006 also in the Stones biotope (only at site EWR 3). For Baetidae this occurred at both sites in the GSM biotope as well as in the Stones biotope at site EWR 3.

When, for example, the Stones biotope of the two sites during 2006 (FLOW phase) is compared with one another it is found that EWR 3 has one species present and EWR 4 has three species present. As discussed in the previous sections of this Chapter, the species generally occur at a site (or are absent from a site) as a result of the habitat and/or flow that are present. When completing the SASS 5 Score Sheet for EWR 3 Baetidae would be marked as having only one species present (sensitivity score = 4) and Baetidae at site EWR 4 would be marked as having >2 species present (sensitivity score = 12). If no additional species were present in the Vegetation and GSM biotopes, it would mean that site EWR 3 would possibly have a lower score than site EWR 4 (resulting from the lower sensitivity score for Baetidae species at the two sites). The interpretation of the lower SASS 5 score would be that this lower score is due to an impact, such as pollution, when in fact the lower score was probably due to the natural changes/differences in the habitat and/or flow at the two respective sites. Thus, in non-perennial rivers with naturally poor habitat and/or relatively low flow, the SASS 5 Score could be low during the FLOW phase and this could be interpreted as the site being degraded, when in fact it is a natural phenomenon in these rivers and should be interpreted as such.

The fact that there were significant differences between the hydrological phases in the species data and not in the family/SASS Score data, implies that using species data could be more successful when assessing river health in non-perennial rivers. This study, using the Seekoei River as a case study, suggests that taxa present in non-perennial rivers are mainly as a result of the available habitat and flow and not necessarily due to pollution, thus, the lower SASS Score is often as a result of natural effects. A study by Watson & Dallas (2013) agrees with this and argued that the SASS users do not always consider the natural flow or habitat availability in non-perennial rivers when interpreting the final SASS 5 score, but rather interpret the lower scores as being due to human influences (unnatural disturbances).

During this study it was found that some species show flow preferences or are only present during flow, while other prefer no-flow. Although it has been proven that families can have hydrological phase preferences (Thirion, 2007; Watson & Dallas, 2013), it seems to be more so at species level. Species hydrological phase preferences especially in non-perennial rivers is therefore an important topic for future research.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

In non-perennial rivers of South Africa the SASS 5 method is not as reliable as in perennial rivers, and this is also stated in Dickens and Graham (2002) who recommend that the SASS 5 method should not be used in non-perennial rivers. The main characteristics distinguishing these river types are, the available habitat and hydrology, therefore this is also believed to be the main factors determining the presence of macroinvertebrates. The available habitat is also influenced by the hydrological phase, thus the main factor investigated was the hydrological phase. This study therefore aims to determine what the impact of the hydrological phase on the macroinvertebrate species composition is and how this influences the SASS 5 methodology, by studying Baetidae and Simuliidae in the Seekoei River.

Some species in the Seekoei River, such as *Cloeon* sp., are more abundant in certain habitats and flow conditions than other. *Cloeon* sp. was found to be the most abundant Baetidae species with preferences for the vegetation biotope and no-flow conditions. Due to their preference for no-flow, *Cloeon* sp. seems to have an even higher preference for the vegetation biotope during the FLOW phase, because of their presence almost only in the vegetation biotope. Species such as *P. vinosum,* and *B. harrisoni* prefer the riffles/rapids in the Seekoei River, either because of the flow present in this biotope or the specific habitat that are found in this biotope. For example, *P. vinosum* prefer the Stones biotope because of their preference for flow conditions, while *B. harrisoni* prefer the rapids for the high availability of cobbles. In short this indicates that although Baetidae are present in both phases, some species occur in higher abundances, or only during certain hydrological phases and certain biotopes.

In the Seekoei River all the Simuliidae species are present only during flow phase, but some species were found to have certain flow preferences and also some that had specific habitat preferences. Species like *S. adersi* are present during all of the flow ranges experienced by the Seekoei River while others such as *S. gariepense* had a preference for very slow to slow flow ranges and *S. damnosum* and *S. hargreavesi* preferred moderate to fast flow. All the Simuliidae species seem to prefer the Stones biotope except *S. gariepense* which rather seems to prefer the GSM and Vegetation biotopes probably due to the lower flow ranges found in these biotopes during the FLOW phase. This emphasises how important the Vegetation biotope can be as a refuge during high flow because flow in the Vegetation biotope was never as high as in the Stones biotope. This also proves that even though the family, Simuliidae, is always present during the FLOW phase, some species only occur during lower flow or higher flow, and some species occur only, or in higher abundances, in certain biotopes.

These changes in species composition as a result of habitat and flow preferences should be considered during the management of non-perennial rivers. When for example, flow is reduced in the Seekoei River above the two sample sites (because of a dam, more weirs, irrigation etc.) it is likely to result in a reduction in species, i.e. *P. vinosum*, with a preference for specific flow conditions. Over time this species can even disappear, reducing the competition and giving pest species like *S. damnosum* (which has the same habitat preferences: Stones and Vegetation biotopes) the opportunity reach pest level abundances. On the other hand, if flow is increased due to the backflow from water purification works, the abundance of species like *Cloeon* sp. will decrease significantly and could also disappear if flow gets too high. Information like this is important during management and would not have been known if only family-level was used.

The SASS 5 method is also demonstrated to be inaccurate in the Seekoei River, because the SASS Score and NoT (families) show no significant differences between the hydrological phases. In other words, the SASS method does not consider the natural changes of the Seekoei River due to its flow regime. This study indicated that when using species-level a significant difference is found between the two phases: FLOW phase and POOLS phase.

Various impacts on rivers can affect the biotope availability, quantity and quality including erosion, agricultural activities, activities decreasing/increasing flow, etc. With some Baetidae and Simuliidae species present only in (or with a preference for) certain biotopes, it is important to preserve all the biotopes. Even though it has been proven in the past that family-level are sufficient, certain important data are lost that is important for managing non-perennial rivers successfully. This indicates just how important the changes at species level are and, therefore proves that it is important to consider species-level rather than family level during the management of non-perennial rivers.

6.2. RECOMMENDATIONS

Although the current study concluded that species data are significantly different between the two hydrological phases, this study should be taken further, especially because the results indicated how important species-level research can be in non-perennial rivers. This study did not include all the families and therefore the other families, especially those from the orders Odonata and Ephemeroptera and eventually all the families, should be considered for potential future research. This type of research is needed for non-perennial rivers in order to establish what species can be present, and what their preferences are in terms of the habitat structure and flow conditions.

Baetidae samples from two years was included in this study, therefore it is also an option for future research to include the other three years (which were sampled as part of the WRC study) to have a larger data set and to determine if the species analyses still give the same results as in this study.

Some Simuliidae species were only present at site EWR 3. This is also something to consider for future research, because it is not clear why. It is believed that the habitat type, especially the long rapid, at site EWR 3 play an important role in this case, but it could be the result of other variables.

All of the above mentioned research is important for management purposes, and future research should expand on the influence of species composition on biomonitoring results. These studies are important because of their contribution to biomonitoring in non-perennial rivers, which is an important topic for future research in non-perennial ecosystems.

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APPENDICES

APPENDIX A: MACROINVERTEBRATE IMAGES

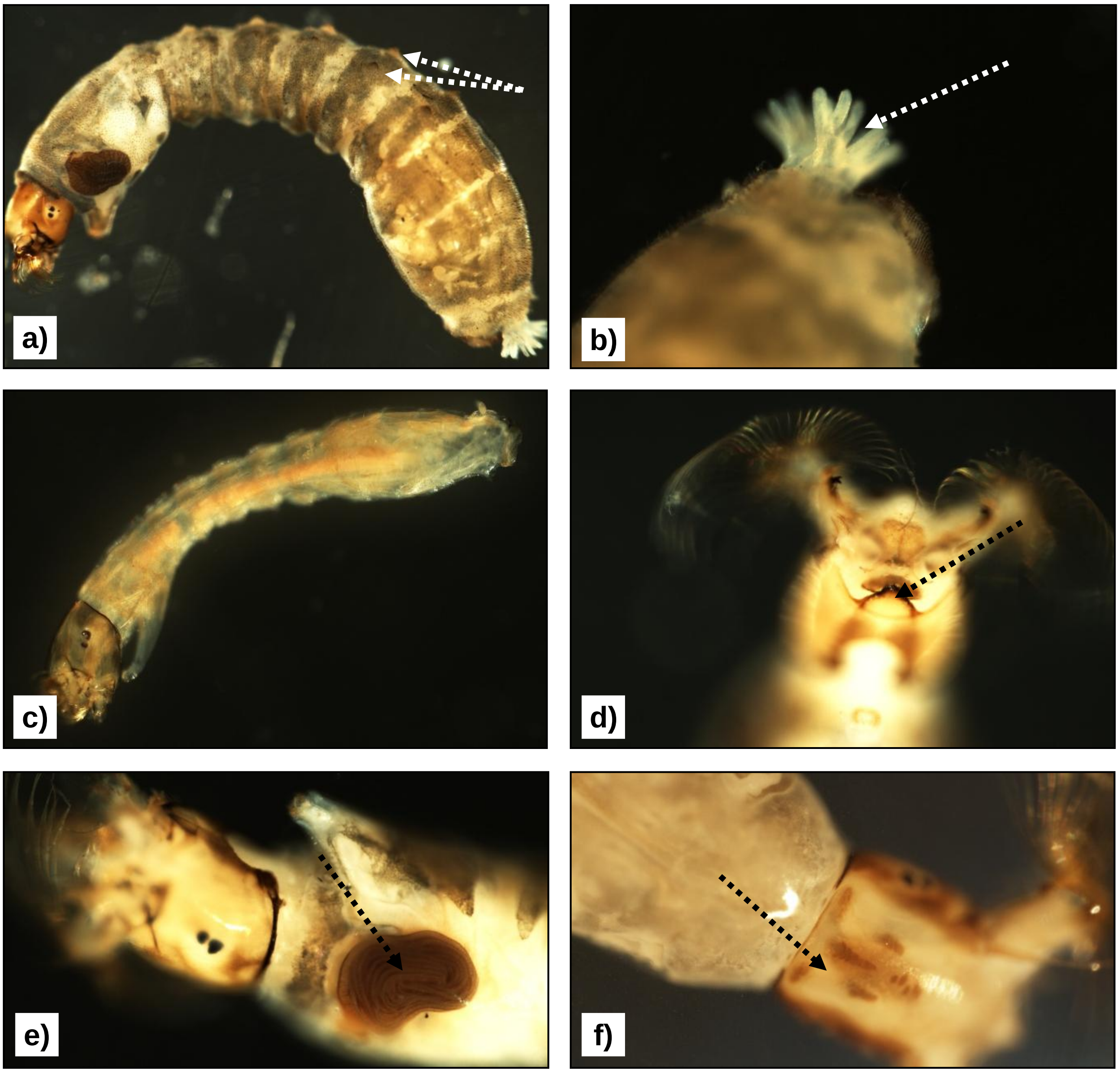

Figure A1: Illustrating certain characteristics for some of the identified Simuliidae species. Larvae of *Simulium damnosum a)* dorso-lateral view indicating paired sub-conical tubercles and setae; and *b)* posterior segments indicating the anal gills. Larvae of *S. adersi c)* the lateral view; *d)* ventral view of head capsule indicating the hypostomium; and e) lateral anterior view indicating the developing gill of the pupae. Larvae of *S. nigritarse* f) dorsal view of head capsule indicating the positive pigmentation pattern.

Figure A2: The labium, ventral view, of the *Cloeon* sp. (Baetidae) identified during this study.

APPENDIX B: ADDITIONAL GRAPHS

An example of plotted line graphs:

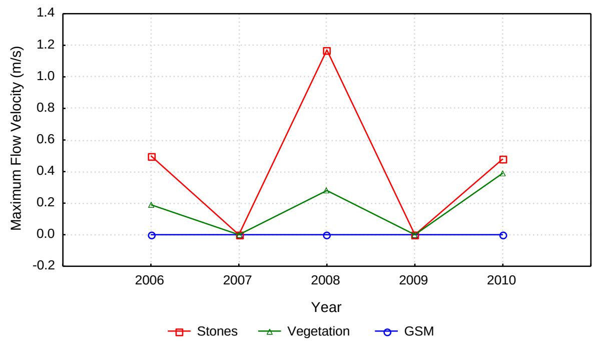

Figure B1: The maximum flow velocity from 2006 to 2010 at site EWR3 in the Seekoei River. Biotopes separated: GSM (Gravel, Sand and Mud), Stones and Vegetation.

Correlations: number of Simuliidae species against maximum flow:

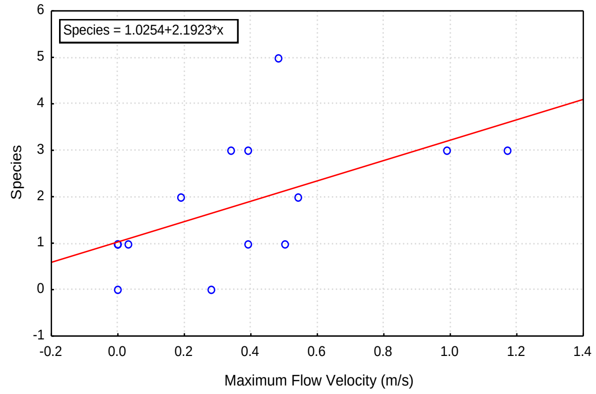

Figure B2: FLOW phase.

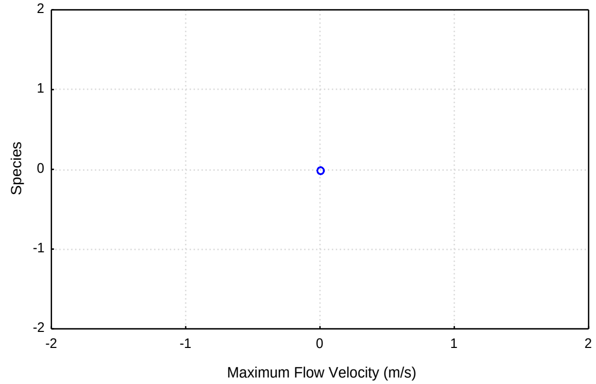

Figure B3: POOLS phase.

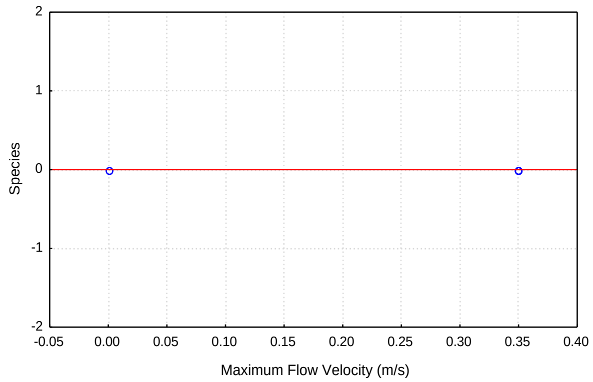

Figure B4: ONSET phase.

APPENDIX C: FULL RESULTS FOR THE STATISTICA ANALYSIS

Table C1: Results for the ANOSIM test between the Groups identified by the Bray Curtis Similarity Cluster in section 5.3.3.

ANOSIM
Analysis of Similarities

One-Way Analysis

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All

Factor Values
Factor: Group
1
2
3

Factor Groups

Sample	Group
306G	1
306V	1
306S	2
406G	2
406V	2
310G	2
410G	2
406S	3
308S	3
310S	3
310V	3
410S	3
410V	3

Global Test
Sample statistic (Global R): 0.956
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from 36036)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
1, 2	0.955	4.8	21	21	1
1, 3	1	3.6	28	28	1
2, 3	0.967	0.2	462	462	1

Outputs
Plot: Graph6
Worksheet: Resem3

Table C2: Results for the SIMPER test between the Groups identified by the Bray Curtis Similarity Cluster in section 5.3.3.

SIMPER
Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data1
Data type: Abundance
Sample selection: All
Variable selection: Al/

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 90.00%

Factor Groups

Sample	Group
306G	1
306V	1
306S	2
406G	2
406V	2
310G	2
410G	2
406S	3
308S	3
310S	3
310V	3
410S	3
410V	3

Group 1
Average similarity: 66.67

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Simulium gariepense	1.00	66.67	#######	100.00	100.00

Group 2
Average similarity: 100.00

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Simulium adersi	1.00	100.00	#######	100.00	100.00

Group 3
Average similarity: 78.48

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Simulium adersi	1.00	32.57	5.62	41.50	41.50
Simulium damnosum	1.00	32.57	5.62	41.50	83.01
Simulium hargreavesi	0.67	11.67	0.78	14.87	97.88

Groups 1 & 2
Average dissimilarity = 66.67

Species	Group 1 Av.Abund	Group 2 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Simulium gariepense	1.00	0.00	41.67	4.74	62.50	62.50
Simulium adersi	0.50	1.00	25.00	0.95	37.50	100.00

Groups 1 & 3
Average dissimilarity = 80.12

Species	Group 1 Av.Abund	Group 3 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Simulium damnosum	0.00	1.00	22.44	4.49	28.01	28.01
Simulium gariepense	1.00	0.00	22.44	4.49	28.01	56.02
Simulium hargreavesi	0.00	0.67	13.83	1.29	17.26	73.28
Simulium adersi	0.50	1.00	12.50	0.92	15.60	88.88
Simulium nigritarse	0.00	0.33	6.33	0.66	7.90	96.78

Groups 2 & 3
Average dissimilarity = 50.00

Species	Group 2 Av.Abund	Group 3 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Simulium damnosum	0.00	1.00	25.00	5.11	50.00	50.00
Simulium hargreavesi	0.00	0.67	15.28	1.34	30.56	80.56
Simulium nigritarse	0.00	0.33	6.94	0.68	13.89	94.44