The determination of flow and habitat requirements for selected riverine macroinvertebrates

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ABSTRACT

The focus of the Department of Water and Sanitation (DWS) has changed from one which addresses the quality and quantity of water resources in isolation to one which integrates these attributes with that of aquatic ecosystem integrity. The right to water for basic human needs as well as to ensure a functioning ecosystem is entrenched in the National Water Act (Act 36 of 1998) through the setting of the Reserve (Rowlston 2011). The Ecological Reserve is defined as the quality and quantity of water required for protecting aquatic ecosystems in order to secure ecologically sustainable development and use of the relevant water resource. Although macroinvertebrates are used to set environmental flows in South Africa and abroad, very limited information is available about their flow requirements (Brunke *et al*. 2001, Schael 2002, Jowett 2002a, Cassin *et al*. 2004, Clifford *et al*. 2004, Hanquet *et al*. 2004, Kleynhans and Louw 2007). In southern Africa some information is available on certain Ephemeroptera in the Inkomati System (Matthew 1968) and some species occurring in the Lesotho Highlands (Skoroszewski and de Moor 1999). A structured approach is required to determine macroinvertebrate environmental requirements taking into account the different life stages, ecoregions, seasonality and substratum.

The purpose of this study was to determine the habitat requirements of selected riverine macroinvertebrate taxa. The main aim was to determine the preferred ranges of water depth, velocity and temperature, as well as the substratum types required by Ephemeroptera, Trichoptera, Coleoptera and Diptera.

In order to determine the habitat requirements for the selected riverine macroinvertebrates, 266 quantitative samples were collected at 52 sites between July 2005 and February 2009. Samples were taken with a Surber sampler and hand net at a number of localities at each site to cover all substratum types, velocity and depth groupings. Basic *in situ* water measurements (temperature, dissolved oxygen, pH and Electric Conductivity) were also collected at each site. The macroinvertebrates were preserved in 80% ethanol and identified to family. The water velocity was also measured at 5 – 10 cm intervals at each locality from as close to the bottom as possible to the water surface.

No comprehensive study has been done on the distribution of aquatic macroinvertebrates in South Africa. The only distribution maps available are those of selected insect families drawn mostly from existing museum and literature records (Picker *et al*. 2003, Griffiths *et al*. 2015). The geographical distribution of 10 Ephemeropteran, 16 Trichopteran, 10 Coleopteran and 14 Dipteran families were determined using data collected from this study as well as archived data

from the National Rivers Database, the Biobase and the National Freshwater Invertebrate Collection housed at the Albany Museum in Grahamstown. The distribution ranges for each of the insect families were then compared to distribution ranges in the literature. The distribution of each of the families was also associated with Level I Ecoregion, geomorphological zone and altitude range. The range extension in a number of taxa such as the Calamoceratidae (Trichoptera) and the Ptilodactylidae (Coleoptera) as well as the identification of questionable distribution records for a number of mostly south-western Cape endemics such as Barbarochthonidae, Sericostomatidae and Glossosomatidae was highlighted. The need to archive voucher specimens, not only for new or unidentified taxa, but also to validate the range distributions of known taxa is emphasised. The scarcity of distribution records for a number of families, most notably that of the Hydrosalpingidae (Trichoptera), Ptilodactylidae, Limnichidae (Coleoptera) and Ephemeridae (Ephemeroptera) is also noted.

Redundancy analysis (RDA) was done using Canoco 5.04 (ter Braak and Šmilauer 2012) to determine the environmental factors contributing most to the distribution of the different invertebrate families. Results from the RDA were then used to draw response curves for the selected families using the Species Response Curve function in Canoco 5.04 (ter Braak and Šmilauer 2012). A Generalised Additive Model (GAM) with Poisson distribution and log link function (family as the response and depth, velocity at 60% of depth and substratum category as predictors) were used with stepwise selection using the Akaike Information Criterion (AIC) and two degrees of freedom (2 DF) factor to smooth the curves. Significance of relationships was regarded as $p < 0.05$.

The concept of Habitat Suitability Curves (HSCs) was developed as part of the Instream Flow Incremental Methodology (IFIM) and the Physical Habitat Simulation System (PHABSIM) in the 1980s by researchers at the United States Fish and Wildlife Service (Bovee 1982, Bovee 1986). Habitat Suitability Curves (HSCs) were determined for the selected taxa using the methods described in Bovee (1986) and Jowett *et al*. (2008). Separate univariate curves were developed for frequency and abundance and the average of the two curves was then used to derive the final HSC. A second order polynomial regression was performed for the depth and velocity curves using Excel 2010. No regression was done on the substratum curves as they represent discrete categories rather than a range of values. Based on the reasoning of Jowett *et al*. (2008), only families with at least twenty individuals and that were present in at least ten samples were selected for the HSCs.

Environmental factors such as velocity, pH, temperature, latitude and longitude, as well as Ecoregion, geomorphological zone and substratum type were determining factors in the distribution patterns of the insect families under consideration. Not all of the factors were important for all of the families. While certain common families (e.g. Baetidae, Chironomidae) showed no preference for any of the environmental factors under consideration, others (e.g. Simuliidae, Blephariceridae) are associated with very fast flowing water over cobbles, the Caenidae with the GSM biotope and Dytiscidae with vegetation. The distribution of taxa with a more limited geographical range such as the more subtropical Calamoceratidae and the burrowing mayflies (Ephemeridae and Polymitarcyidae) are associated with Ecoregion as well as latitude and longitude while the distribution of the southwestern Cape endemic mayflies (Teloganodidae) and cased caddisflies (Sericostomatidae, Glossosomatidae) are also associated with low pH values. The importance of noting the developmental stage of insects such as larva, pupa and adult is highlighted most notably by the different environmental requirements of the beetles where the larval and adult stages sometimes have different requirements.

These results provide a first step in setting habitat requirements for selected families of Ephemeroptera, Trichoptera, Coleoptera and Diptera, and the need for more data on certain families (Prosopistomatidae, Sericostomatidae, Glossosomatidae, Haliplidae, Ephydridae, and Syrphidae) is pointed out. Although depth does not appear to be a determining factor in the occurrence of the macroinvertebrate families investigated here, there is still a requirement for investigating the effect of particularly shallower depths as there might be a threshold value below which the macroinvertebrates could potentially be affected. There is a real danger of damaging the riverine macroinvertebrate communities if depth is ignored and the focus is solely placed on substratum and velocity as there can still be water of a suitable velocity but the depth might be too shallow to enable long-term survival of the resident macroinvertebrates.

The Macroinvertebrate Response Assessment Index (MIRAI) was developed as part of a suite of EcoStatus indices to be used in the Ecological Classification Process (Thirion 2007). The MIRAI is based on the principle that macroinvertebrates integrate the effect of the modification of drivers (hydrology, geomorphology and physico-chemical conditions). The degree of change from natural is rated on a scale of 0 (no change) to 5 (maximum change) for a variety of metrics. Each metric is weighted in terms of its importance to determining the Ecological Category under natural conditions for the specific locality. The main aim of the Ecological Classification process is to acquire a better understanding of the reasons for the system’s deviation from the natural or reference condition. The distribution of an aquatic macroinvertebrate assemblage is determined by the tolerance of the individuals in the population to an array of environmental factors. It is therefore essential that all habitat features are considered when evaluating the suitability of habitat for aquatic macroinvertebrates. The approach followed in assessing macroinvertebrate response to driver characteristics is based on a qualitative combination of information gained by field surveys, the available habitat as a result of driver conditions, and the traits of the macroinvertebrates present (Lamaroux *et al*. 2004, Horta *et al*. 2009).

The Habitat Suitability Curves (HSCs) were converted to values out of 5 and rounded to the nearest 0.5 to fit in with the system used in the suite of EcoStatus models. Where no or not enough data were available, information from the literature was used to assign the preference values to each taxon. Teresa and Casatti (2013) suggested that values greater than 0.7 can be regarded as preferred conditions. It was therefore decided to use preference values greater than 3.5 to indicate a strong preference for a certain habitat feature. No changes were made to the physico-chemical (water quality) metric group as these ratings are based on the sensitivity values (QVs) used in the South African Scoring System (SASS) version 5 (Dickens and Graham 2002). The preference values in MIRAI v1 were then updated to reflect these new values. This new version (MIRAI v2) was then tested by running it for 44 sites covering a large geographical range and ecological conditions. The results from the two different versions of MIRAI were compared using a one-way analysis of variance (ANOVA) and a linear regression analysis done using Excel 2010.

The changes to the velocity and substratum preference ratings in the MIRAI model resulted in only small changes to the total MIRAI score. The MIRAI category generally remained the same or at most changed by half a category. The largest percentage change was 5.7% at the Sterkstroom (Site A2STER-MAMOG) that remained in a D Category, and 5.3% at the Jukskei River (Site A2JUKS-DIENR) that changed from a DE category to an E category for MIRAI v2. The relatively small changes in MIRAI, together with the high correlation values (>0.9) means that the results from the two versions should be compatible and no adjustments will be needed to the results obtained from the original MIRAIs. The impact of the larger changes to the Flow modification and Habitat modification metric group will however need to be investigated.

The following three hypotheses were tested in this study;

- The macroinvertebrate assemblage structure can be differentiated based on Ecoregion delineation and geomorphological zonation. The results indicated that this is true for certain taxa while other taxa have a countrywide distribution and have been recorded from most geomorphological zones. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on Ecoregion and geomorphological zone. This hypothesis is thus accepted.

- The macroinvertebrate assemblage structure can be differentiated based on environmental factors such as substratum, depth and velocity as well as physico-chemical parameters. The results indicated that this is true for certain taxa but not for others. It is also clear that some of the environmental factors play a role in the distribution of certain taxa but not others (e.g. temperature is a determining factor for Blephariceridae, but not for Simuliidae). Depth was not a significant factor in determining the distribution of the insect families under consideration. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on a combination of environmental factors. This hypothesis is therefore accepted.

- The different habitat requirements of the macroinvertebrate taxa in terms of velocity and substratum type can be used to refine the macroinvertebrate taxa's preference values in the Macroinvertebrate Response Assessment Index (MIRAI), to assess the ecological condition of the macroinvertebrate assemblage. The preference ratings, based on the HSCs as well as information from the literature and personal experience, were successfully used in MIRAI v2 to determine the ecological condition of the macroinvertebrate assemblage at 44 sites spanning a range of conditions. The high correlation values (>0.9) for the different MIRAI metrics tested clearly indicates that the hypothesis can be accepted.

The following recommendations were made:

- Information collected post January 2014 should be used to update the distribution ranges. The updated distribution maps and associated KML files should be placed on the RQIS website.
- Gaps identified should be filled by actively targeting areas with limited or no data and a concerted effort made to collect information on the distribution of taxa with limited records.
- A sampling programme should be designed and implemented to include the water surface as a possible habitat and the study area expanded to include polluted sites as well.
- HSCs and preference ratings should be developed for taxa not included in this study.
- Specimens should be identified to genus or species level and HSCs and preference ratings for these genera or species determined where possible.
- A wider range of depths should be included in order to determine if there is a minimum depth requirement that should be used when determining Environmental Water Requirements.
- Information obtained should be included in the RHAMM and FIFHA models.
- A list of possible reference taxa per Level II Ecoregion, geomorphological zone and altitude range should be compiled based on information obtained during this study. These lists should be included in MIRAI v2 as well as the RHAMM model. This will enable a user to compile a reasonable reference condition for a site, based not only on the list but also on the natural characteristics of the site. Ideally these reference conditions should be placed in a central location such as the RQIS websites where other researches can access it.
- The effects of the changes in the flow modification and habitat modification metric group results between the two MIRAI versions should be investigated and the following questions answered:
    - Does it have an impact on the explanation for the Ecological condition/ impacts at the site?
    - Does it explain the impacts more realistically than the information obtained using MIRAI v1?

Key Terms:

Freshwater Invertebrates

Habitat modification

Ecological Integrity

Flow modification

Environmental Water Requirements

Geographical distributions

Ecoregion

Geomorphological Zonation

Reference Conditions

Habitat Suitability Curves

MIRAI: Macroinvertebrate Response Assessment Index

EcoStatus: Ecological Status

RHAMM: Rapid Habitat Assessment Method and Model

RIVDINT: River Data Integration System

FIFHA: The Fish Invertebrate Habitat Assessment Model

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

In situations where there is an ample supply of water and a small population it is a reasonably simple matter to obtain enough water for domestic, agricultural and industrial use. However, increasing demand on our water resources necessitates the active management of water to ensure equitable distribution. In South Africa this has led to the damming of rivers for domestic, industrial, agricultural and hydro-electrical purposes but also to the implementation of Inter-basin transfer schemes on a large scale (King *et al*. 2011). South Africa's water problems are exacerbated by a below world average rainfall that is spread very unevenly throughout the country and throughout time. The flow regimes of South Africa and Australia were found to be amongst the most variable in the world (McMahon *et al*. 2007a, McMahon *et al*. 2007b). The eastern- and southern coastal areas of South Africa have considerably higher rainfall than the interior and western coastal areas (Department of Water Affairs and Forestry 2008).

Changes to the Constitution of the Republic of South Africa have altered the focus of the Department of Water and Sanitation (DWS) from one which addresses the quality and quantity of water resources in isolation, to one which integrates these attributes with that of aquatic ecosystem integrity. The National Water Act (Act 36 of 1998) recognises two water rights that are protected through the setting of the Reserve. The Reserve ensures the availability of water for basic human needs as well as for ecological requirements. The Ecological Reserve is defined as the quality and quantity of water required for protecting aquatic ecosystems in order to secure ecologically sustainable development and use of the relevant water resource (Rowlston 2011).

Changes in the flow regime have a potential impact on freshwater ecosystem integrity. These changes are due, among others, to abstraction of water, and release of water into a system often as a result of economic development. In response to the ecological consequences of diminishing and altered flow regimes, a range of methods have evolved that attempt to quantify the Instream Flow Requirements (IFR) of rivers (Pollard 2002).

CHAPTER 1

Freshwater macroinvertebrates have been used to assess the biological integrity of stream ecosystems with relatively good success throughout the world (Rosenberg and Resh 1993, Resh *et al*. 1995, Barbour et al. 1996, Blackburn and Mazzacano 2012), more commonly than any other biological group (O'Keeffe and Dickens 2000) because they offer a good reflection of the prevailing flow regime and physico-chemical conditions in a river. In addition they form an essential component of the riverine ecosystem (Allan 1995, Skoroszewski and de Moor 1999, O' Keeffe and Dickens 2000, Weber *et al*. 2004). Freshwater macroinvertebrates are important processors of transported organic matter in rivers, serve a vital function in purifying the water in a river, and also provide a valuable food source for larger animals within and even outside the system (Allan 1995, Skoroszewski and de Moor 1999, O' Keeffe and Dickens 2000, Weber *et al*. 2004).

The distribution of a freshwater macroinvertebrate population is determined by the physico-chemical tolerance of the individuals in the population to an array of environmental factors (Cummins 1993). The distribution pattern resulting from habitat selection by a given freshwater macroinvertebrate species reflects the optimal overlap between habitat (mode of existence) and physical environmental conditions that comprise the habitat, substrate, flow and turbulence factors (Cummins 1993).

Habitat functions as a temporally and spatially variable physical, chemical, and biological template within which aquatic invertebrates can occur (Orth 1987, Poff and Ward 1990,). Numerous studies have demonstrated the importance of physical habitat quantity and quality in determining the structure and composition of biotic communities (e.g. Modde *et al*. 1991, Aadland 1993, Ebrahimnezhad and Harper 1997). Habitat can also be defined as any combination of velocity, depth, substrate, physico-chemical characteristics and biological features that will provide the organism with its requirements for each specific life stage at a particular time and locality (Bovee 1982).

Populations of benthic animals reflect the microenvironment on a scale smaller than the riverbeds of pools and riffles and also reflect the topographic features of rivers and the effects of improvement works among others on the river environment (Yabe and Nakatsugawa 2004). Suitable environmental conditions and resources (quantity, quality and timing) have to be available in order to sustain a viable long-term population (Colwell and Futuyma 1971, May and

MacArthur 1972, Pianka 1974, Statzner and Higler 1986). Because a variety of factors such as environmental conditions and resources are required to meet the life history requirements of species, the success of aquatic organisms can be limited by a single factor or by a combination of factors (Hardy 2000).

Since many aquatic organisms have specific habitat requirements, seasonal variation in these factors may lead to seasonal variation in the distribution and abundance of benthic macro-invertebrates (Jacobson 2005, Bogan and Lytle 2007, Fourie *et al.* 2014). Variation in discharge often translates into differences in wetted perimeter, hydraulic conditions and biotope (portion of a habitat associated with a specific assemblage) availability (King *et al*. 2000, O'Keeffe *et al*. 2002, King *et al*. 2004, James and Suren 2009). For example, biotopes such as runs become riffles under low-flow conditions, and marginal vegetation may change from lotic to lentic (Dallas 2004a). Temperature often varies with season and the life cycles of many aquatic organisms are cued to temperature (Kosnicki and Burian 2003, Dallas 2004a). Temperature may also affect the rate of development, reproductive periods and emergence time of organisms (Hawkins *et al*. 1997, Kosnicki and Burian 2003). All organisms have a range of temperatures within which optimal growth, reproduction and general fitness occur, and temperatures outside this range may lead to the exclusion of taxa unable to tolerate such extremes (Coutant 1977, Hawkins *et al*. 1997, Lessard and Hayes 2003, Caissie 2006).

The need to more closely integrate physical and biological function in river systems at a variety of scales has recently been emphasised in the eco-hydraulics literature (Clifford *et al*. 2004). One way of combining the physical and biotic environments within rivers is to classify velocity-depth and velocity-substratum combinations into so called biotopes such as riffles, runs and pools (Clifford *et al*. 2004). In South Africa, Kleynhans and Thirion (2015b) have introduced this concept in the development of the Rapid Habitat Assessment Method and Model (RHAMM). Although it is relatively easy to identify these biotopes in the field it is quite complicated to relate them to biotic function in a meaningful way (Clifford *et al*. 2004). Due to the unique physical and chemical characteristics of the different biotopes, they support different combinations of plants and animals. The changes to riverine habitats imply that there must be transition zones or ecotones. Although some of these zones are fairly stable, others such as the sediments are very dynamic especially as a result of changes in the flow regime (Gonzalez *et al*. 2004).

Although macroinvertebrates are used to set environmental flows in South Africa and abroad, very limited information is available about their flow requirements (Brunke *et al*. 2001, Schael 2002, Jowett 2002a, Cassin *et al*. 2004, Clifford *et al*. 2004, Hanquet *et al*. 2004, Kleynhans and Louw 2007, Thirion 2007). In southern Africa some information is available on certain Ephemeroptera in the Inkomati System (Matthew 1968) and some macroinvertebrate species occurring in the Lesotho Highlands (Skoroszewski and de Moor 1999). A more structured approach is required to determine macroinvertebrate environmental requirements taking into account the different life stages, ecoregions, seasonality and substratum. A pilot project (Zituta 2002) to address the flow requirements of different Baetidae species occurring in the Elands and Sabie rivers was unfortunately not completed.

This thesis focuses on the ecological requirements of four insect orders commonly used during the determination of the Ecological Reserve (Skoroszewski and de Moor 1999, Skoroszewski 2006, Brown *et al*. 2009). The four orders chosen are: Ephemeroptera, Trichoptera, Coleoptera and Diptera as they are believed to contain the most sensitive rheophilic macroinvertebrate taxa (Blackburn and Mazzacano 2012).

1.2 Hypotheses, aims and objectives

The purpose of this project is to determine the habitat requirements of selected macroinvertebrate taxa. It will aim to determine the preferred ranges of water depth, velocity and temperature, as well as the substratum types required by a number of different macroinvertebrate taxa. It is envisaged that the results from this project will assist in the setting of the ecological (flow) component of the Ecological Reserve.

In this study three hypotheses will be tested:

1) The macroinvertebrate assemblage structure can be differentiated based on Ecoregion delineation and geomorphological zonation;
2) The macroinvertebrate assemblage structure can be differentiated based on environmental factors such as substratum, depth and velocity as well as physico-chemical parameters;

3) The different habitat requirements of the macroinvertebrate taxa in terms of velocity and substratum type can be used to refine the macroinvertebrate taxa's preference values in the Macroinvertebrate Response Assessment Index (MIRAI), to assess the ecological condition of the macroinvertebrate assemblage.

The objectives of the project are as follows:

- Identify and describe the macroinvertebrate communities found at selected study sites;
- Describe the geographical range (Level II ecoregion and geomorphological zone of each of the taxa);
- Identify the environmental requirements (range of occurrence as well as preferred ranges) of each taxon in terms of water depth, current velocity and substrate composition;
- Use these results to update the Macroinvertebrate Response Assessment Index (MIRAI) developed as part of the suite of EcoStatus models (Thirion 2007).

1.3 Structure of the thesis

Chapter 1 presents the need and context of this study together with the aim, hypotheses, and objectives. In Chapter 2 the geographical distribution of 10 Ephemeropteran, 16 Trichopteran, 10 Coleopteran and 14 Dipteran families are presented. The distribution of the selected insect families is then related to Level II Ecoregions, geomorphological zones and altitude. The habitat requirements of six of the Ephemeropteran, five of the Trichopteran, seven of the Coleopteran and eight of the Dipteran families with regards to water depth, velocity and substratum type is then determined in Chapter 3. The information generated in Chapter 3, is used in Chapter 4 to determine velocity and substratum preference ratings for the selected macroinvertebrates. Information from the literature is used to determine the preference ratings for taxa where no or not enough data was available to calculate Habitat Suitability Curves (HSCs). These ratings are then used to update the Macroinvertebrate Response Assessment Index (MIRAI). The original and updated version of the MIRAI is then run for a range of sites and the results obtained from the two versions compared to each other.

CHAPTER 2

THE GEOGRAPHICAL DISTRIBUTION OF EPHEMEROPTERA, TRICHOPTERA, COLEOPTERA AND DIPTERA IN SOUTH AFRICA.

2.1 Introduction

No comprehensive study has been done on the distribution of freshwater macroinvertebrates in South Africa. The only distribution maps available are those of selected insect families drawn mostly from existing museum and literature records (Picker *et al*. 2003, Griffiths *et al*. 2015). The distribution maps in Picker *et al*. (2003) and Griffiths *et al*. (2015) give a broad indication of where the insects are likely to occur but are not intended to indicate precise and total distribution. Extensive hydrobiological surveys of a number of major river systems were conducted by the Council for Scientific and Industrial Research (CSIR) and National Inland Water Research programme (NIWR) between 1958 and 1975 with most of the collected material lodged in the Albany Museum (de Moor 1992). These studies focused on certain areas of the country (Harrison 1959, Oliff 1960, Harrison and Agnew 1962, Oliff 1963, Oliff and King 1964, Harrison 1965, Oliff *et al*. 1965, Schoonbee 1973), or on certain taxa only (Barnard 1934, Agnew 1962). There were only a few more recent studies done (Moore 1991, Palmer 2000, Brown 2001, Dallas 2002, Madikizela and Dye 2003, Nunkumar 2003, Schael and King 2005, Bonada *et al*. 2006), but these were all limited in extent. The most comprehensive study was by Harrison and Agnew to determine 12 Hydrobiological regions (Harrison 1958, Agnew and Harrison 1960a, Agnew and Harrison 1960b, Agnew and Harrison 1960c, Harrison and Agnew 1960, Agnew 1961). These studies (Harrison 1965, Scott 1988) found that southern African riverine macroinvertebrates can be divided into two main groups with sub-groups. These groups are:

1) A south temperate, cool-adapted Gondwana element representing cold stenothermal, mostly montane fauna. Although these taxa are mostly found in the western Cape, they may extend in an easterly direction along the southern- and eastern Cape mountain ranges and in a few cases to the KwaZulu-Natal and Mpumalanga Drakensberg. These taxa include the three endemic caddisfly families (Hydrosalpingidae, Petrothrincidae and Barbarochthonidae) as well as some Sericostomatidae genera among others.
2) A Pan-Ethiopian element constituting the bulk of the fauna comprising:

a. A few remaining elements of the tropical Gondwana fauna such as the Pisuliidae caddisflies as well as some Hydropsychidae and Polycentropodidae with eastern affinities;
b. Elements that have mainly entered from the north and some from the east, including Palearctic and Oriental taxa. Many of these taxa have a very wide cosmopolitan distribution.

Historically, distribution records of aquatic insects were frequently constructed largely from collections of the terrestrial adult stages (Sutcliffe 2003). The adult aquatic insects are however, more likely to disperse and are usually more ephemeral (Hynes 1984, Sheldon 1984) with the exception of the truly aquatic Coleoptera. Larval specimens provide a more accurate indication of where species are breeding and spend the majority of their lives. However, the use of larval specimens has the disadvantage that the information on taxonomy is often lacking and many recognised larval types have not been associated with adult species (Sutcliffe 2003). The start of more systematic surveys of South African rivers with the development of the South African Scoring System (Chutter 1998) and the subsequent development of the National Rivers Database (Department of Water Affairs and Forestry 2007) to store the data, allows the development of more comprehensive distribution maps of the freshwater macroinvertebrates at least on a family level.

This chapter describes the distribution of the families within the orders of Ephemeroptera, Trichoptera, Coleoptera and Diptera occurring in South Africa. Data were obtained from samples collected for this thesis, the Rivers Database (which stores information collected during biomonitoring surveys using the South African Scoring System) as well as from the Biobase database (Dallas *et al*. 1999). In addition to the data available from the National Rivers Database, distribution records of the Coleopteran family Ptilodactylidae, that is not included on the SASS datasheet but which has been recorded regularly in the western and southern Cape, were obtained from regional staff of the Department Water and Sanitation in the Western- and Eastern Cape. Historical records from the Freshwater Invertebrate Collection were also obtained from the Albany Museum in Grahamstown (Albany Museum Grahamstown 2014). Because the majority of the information are from the National Rivers Database only families that form part of SASS, with the exception of Ptilodactylidae, have been included.

Historical collections of aquatic macroinvertebrates in South Africa, as elsewhere in the world, have been sporadic and patchy with some locations thoroughly sampled and others not at all. The map indicating the sampling sites (Figure 2.1) used for this chapter indicates that the spread of sites cover the majority of South Africa with only the very dry regions in the Karoo, Northern Cape and extreme north western areas with limited sites.

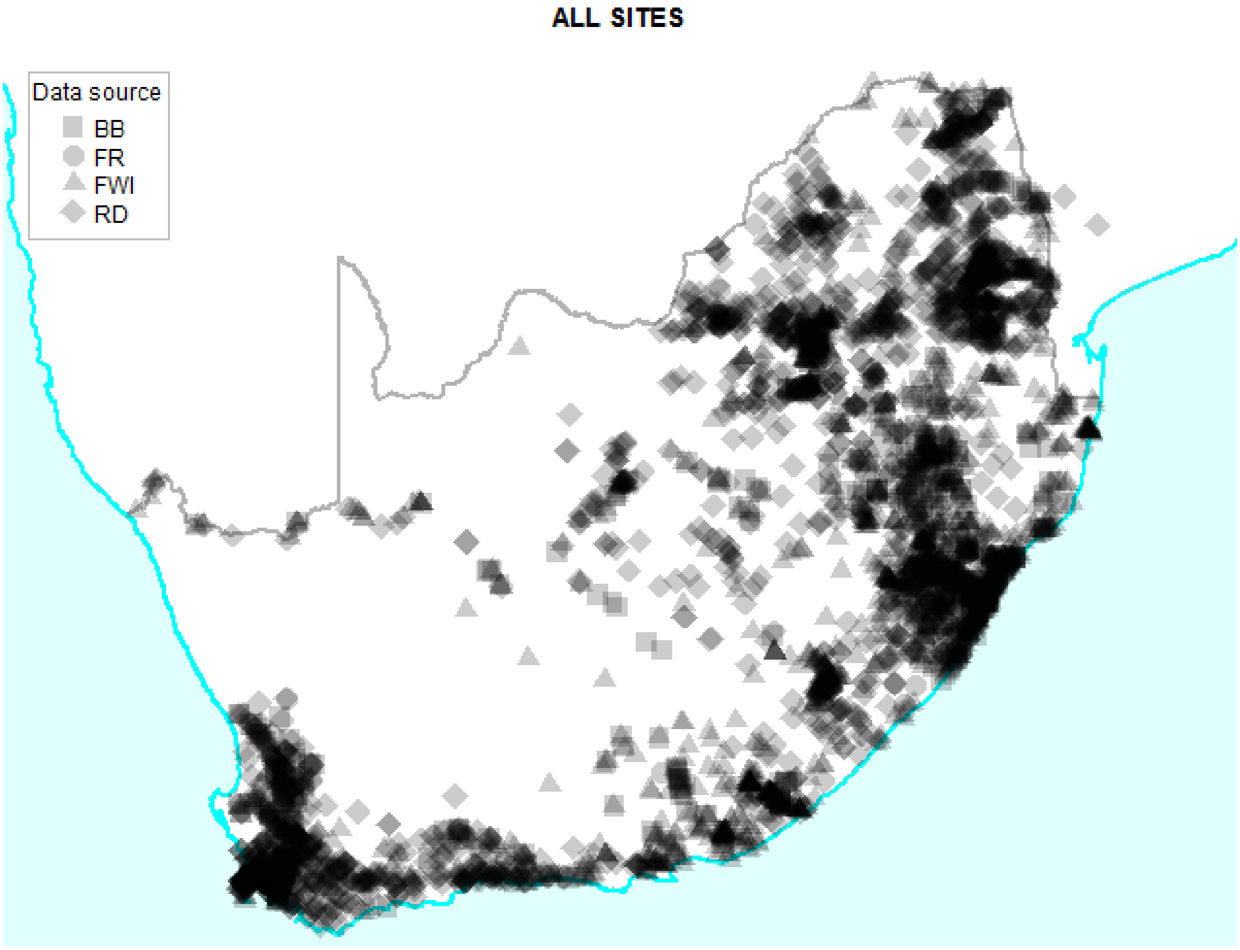

Figure 2.1: A map indicating the monitoring sites used in this study. The different symbols indicate the four data sources used in this study: The Biobase (BB), Environmental Requirements of Aquatic Invertebrates (FR), Freshwater invertebrate collection (FWI) and Rivers Database (RD).

2.2 Methods

The results from the Albany Museum database (AMGS) were filtered to extract only results from South Africa, Lesotho and Swaziland. All data records that were not geo-referenced were excluded. The remaining records were then plotted on Google Earth to ensure that all records plotted within the boundaries of South Africa, Lesotho and Swaziland. The locations outside the borders of South Africa, Lesotho and Swaziland were checked against the descriptions and the coordinates were corrected, where possible. Examples of problems with the geo-referencing included the transposing of latitudes and longitudes as well as excluding the degree and then using the minute value to calculate the decimal degrees. The remaining sites were plotted on a map of South Africa indicating the source of the different sites (Figure 2.1). The results from the different sources were filtered on Excel 2010 for each of the families and duplicate sites were removed. A script written in R (R Core Team 2013) with packages XLConnect (Mirai Solutions GmbH 2013) and maptools (Bivand and Lewin-Koh 2013) was used to read spreadsheets containing family data and produce distribution maps. Transparent symbols give an idea of data density: where many records were available, the overlapping symbols are darker. The shading on the maps indicating the distribution range was then drawn in by hand on Microsoft PowerPoint.

2.3 Results and discussion

The updated distribution maps of the 50 families in the four orders are presented in Figures 2.2 – 2.5. The different data sources used in this study are indicated by the following codes on the maps: Biobase (BB), Rivers Database (RD), Albany Museum Records (FWI) and the Invertebrate Flow Requirements Study (FR). The only level I Ecoregion not sampled is the Namaqua Highlands (Kleynhans *et al*. 2005b) located in the dry western part of the country. There were sites in all the geomorphological zones associated with the normal profile described in Rowntree *et al*. (2000). The geomorphological zones were obtained from Google Earth Overlays created from Moolman (2008).

2.3.1 Ephemeroptera

According to Barber-James and Lugo-Ortiz (2003), there are 11 families, 47 genera and 102 mayfly species in South Africa; although the number of genera and species were expected to increase as the mayfly fauna of the region is more thoroughly documented. Barber-James and Lugo-Ortiz (2003) consistently found genera previously known to occur north of the Cunene and Zambezi Rivers to extend their ranges to Southern Africa. Table 2.1 indicates the distribution of the Ephemeroptera families per Level I Ecoregion and Geomorphological zones, as well as the altitude ranges for each of the families in 500 m intervals.

The Baetidae are widespread throughout the world except in New Zealand and other remote oceanic islands (Barber-James and Lugo-Ortiz 2003). Baetid nymphs are generally found in flowing waters although certain genera (e.g. *Cloeon* and *Procloeon*) are found in still waters and temporary water bodies (Barber-James and Lugo-Ortiz 2003). The family is found throughout the whole of South Africa (Figure 2.2 a, Table 2.1). The Baetidae occurred in all sampled ecoregions and geomorphological zones up to altitudes of 3500 m above mean sea level (a.m.s.l). The distribution shows a broader distribution than in Picker *et al*. (2003) in the sense that there are also distribution records in the Orange-Vaal system that are not indicated in Picker *et al*. (2003). On the other hand, Picker *et al*. (2003) indicates this family's presence in the very dry section of the Kalahari (Western border with Namibia) where there is virtually no surface water at all (Figure 2.2 a).

The Caenidae occur worldwide, but like most mayflies are not found on oceanic islands (Barber-James and Lugo-Ortiz 2003). Caenidae are poorly known from Africa with only three genera recognised in South Africa. According to Picker *et al.* (2003), the Caenidae have a widespread distribution in South Africa (Figure 2.2 b) with virtually the same distribution pattern as the Baetidae. The Baetidae and Caenidae have similar distribution patterns with Caenidae also occurring in all sampled ecoregions and geomorphological zones up to altitudes of 3500 m a.m.s.l (Table 2.1, Figures 2.2 a, b).

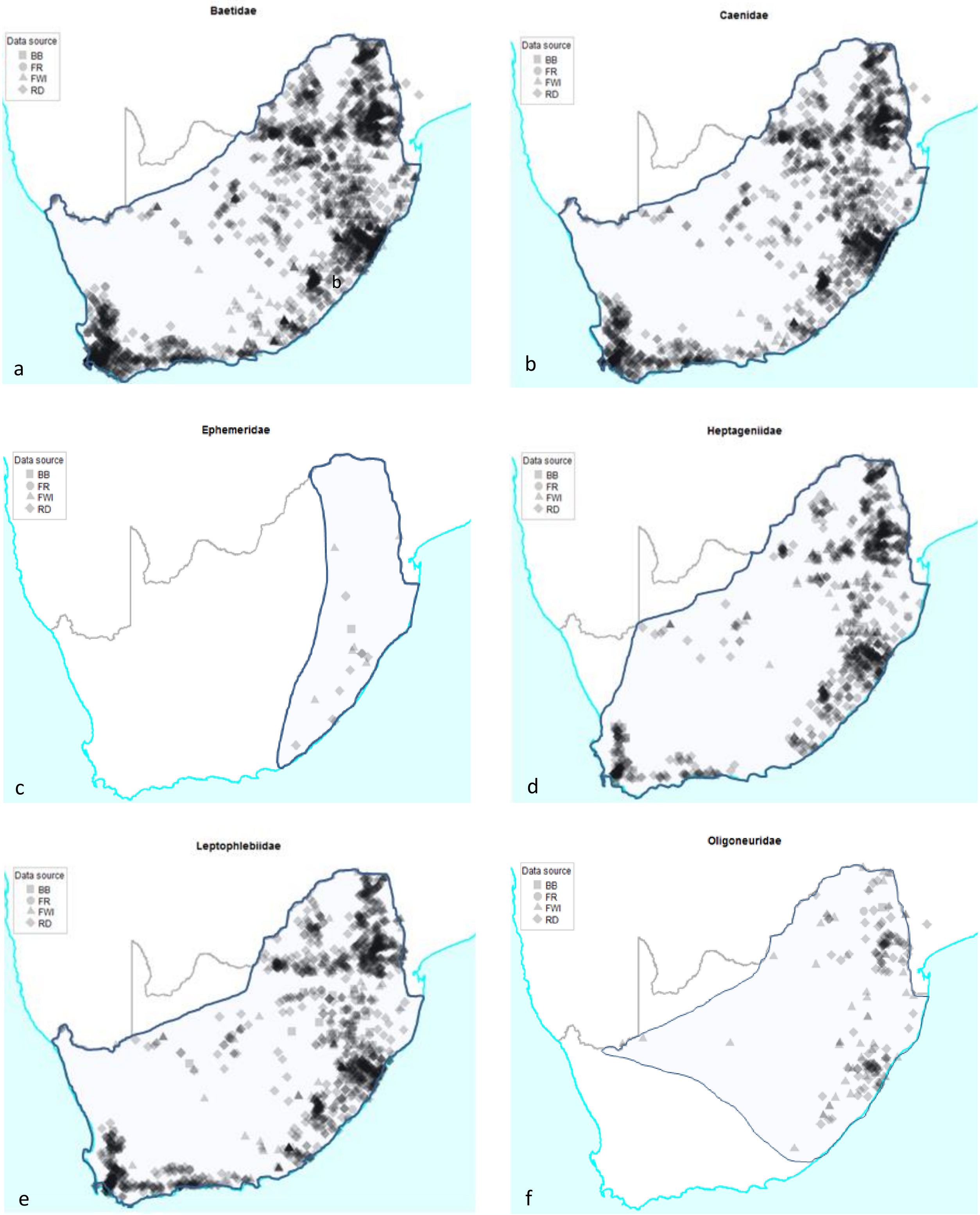

Figure 2.2: Distribution map of Ephemeroptera in South Africa: (a) Baetidae, (b) Caenidae, (c) Ephemeridae, (d) Heptageniidae, (e) Leptophlebiidae, (f) Oligoneuriidae, (g) Polymitarcyidae, (h) Prosopistomatidae, (i) Teloganodidae, (j) Tricorythidae

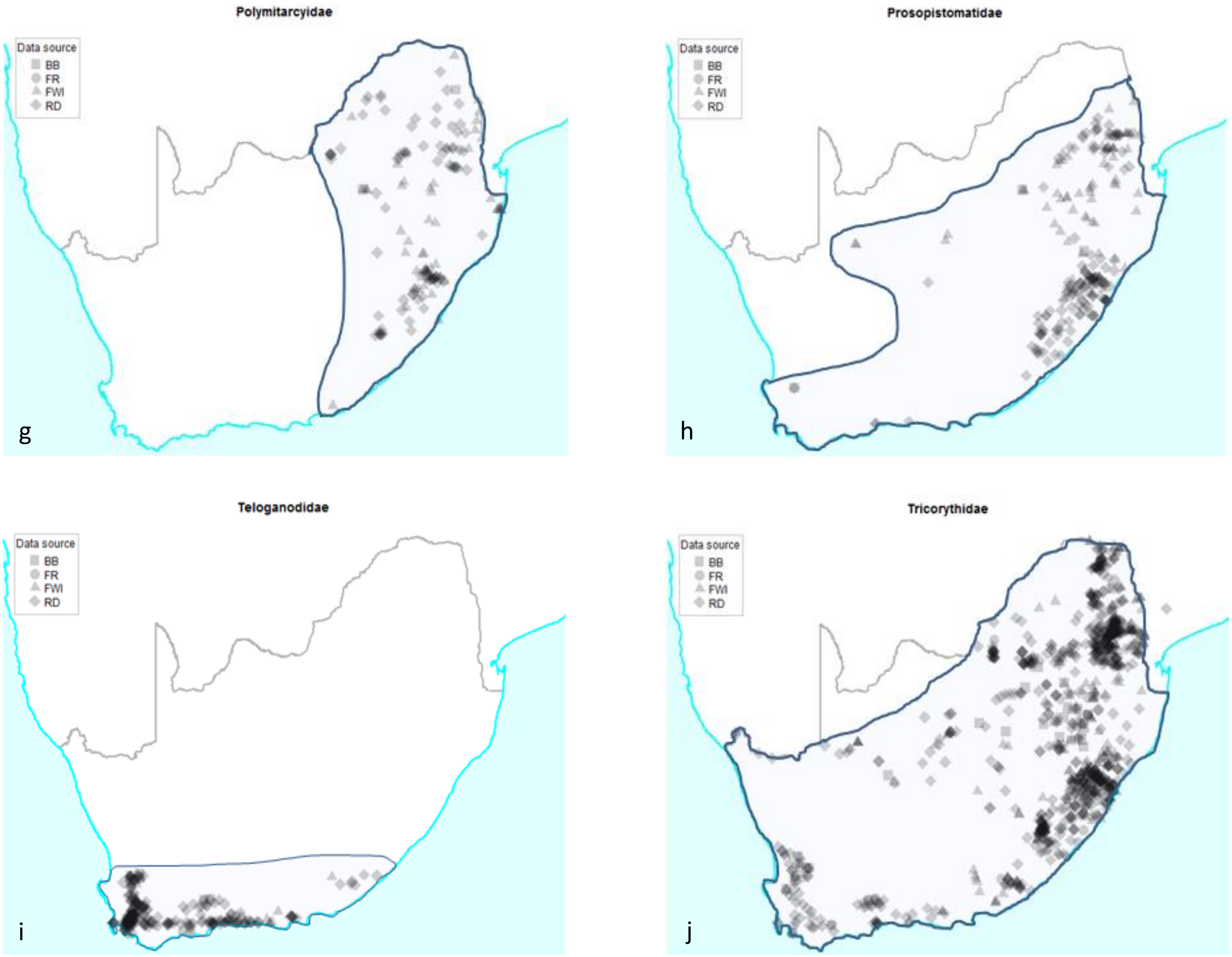

Figure 2.3 (continued): Distribution map of Ephemeroptera in South Africa: (a) Baetidae, (b) Caenidae, (c) Ephemeridae, (d) Heptageniidae, (e) Leptophlebiidae, (f) Oligoneuriidae, (g) Polymitarcyidae, (h) Prosopistomatidae, (i) Teloganodidae, (j) Tricorythidae

The Ephemeridae are found on all continents except Australia. There are three genera in South Africa (Barber-James and Lugo-Ortiz 2003). Very little information is available on the distribution of Ephemeridae in South Africa as this family is very rarely found during biomonitoring surveys. According to Agnew (1985), the nymphs burrow in the muddy bottoms of large tropical rivers thereby limiting their distribution to the north-eastern part of South Africa. The few locations where the Ephemeridae have been recorded are for the most part limited to KwaZulu-Natal (KZN), the Pondoland area in the Eastern Cape and the Mpumalanga Lowveld (Figure 2.2 c). One of the seven records in the Freshwater Invertebrate collection (Albany Museum Grahamstown 2014) was collected from the Ngagane River in KZN but no coordinates were given and it could therefore not be included on the map. Ephemeridae were only recorded in the foothill and lowland geomorphological zones and at altitudes up to 2000 m a.m.s.l (Table

2.1). Despite considerable effort into finding this family for a separate project to photograph live specimens, not a single specimen was sampled and there is therefore doubt regarding the records on the Rivers Database. It is possible that at least some of the specimens from the Rivers Database might in fact have been the more commonly occurring Polymitarcyidae rather than Ephemeridae.

Table 2.1: The distribution ranges of Ephemeroptera families based on distribution records obtained from the Biobase, Rivers Database, Freshwater Invertebrate Collection and a study to determine the environmental requirements of four different Insect orders.

	Ephemeroptera Family	Baetidae	Caenidae	Ephemeridae	Heptageniidae	Leptophlebiidae	Oligoneuridae	Polymitarcyidae	Prosopistomatidae	Teloganodidae	Tricorythidae
	Altitude Range (m a.m.s.l.)	0-3500	0-3500	0-2000	0-3000	0-3500	0-3000	0-2500	0-2500	0-1000	0-3000
ZONE	Source zone	X				X					
	High-gradient Mountain Stream	X	X		X	X	X		X	X	X
	Mountain Stream	X	X		X	X	X	X	X	X	X
	Transitional Zone	X	X		X	X	X	X	X	X	X
	Upper Foothills	X	X	X	X	X	X	X	X	X	X
	Lower Foothills	X	X	X	X	X	X	X	X	X	X
	Lowland	X	X	X	X	X	X	X	X	X	X
LEVEL I ECOREGION	Limpopo Plain	X	X		X	X	X	X			X
	Soutpansberg	X	X		X	X	X	X			X
	Lowveld	X	X		X	X	X	X	X		X
	North Eastern Highlands	X	X		X	X	X	X	X		X
	Northern Plateau	X	X			X					X
	Waterberg	X	X		X	X	X	X			X
	Western Bankenveld	X	X		X	X	X	X			X
	Bushveld Basin	X	X		X	X	X	X			X
	Eastern Bankenveld	X	X	X	X	X	X	X	X		X
	Northern Escarpment Mountains	X	X		X	X	X	X	X		X
	Highveld	X	X		X	X	X	X	X		X
	Lebombo Uplands	X	X		X	X	X	X	X		X
	Natal Coastal Plain	X	X		X			X			
	North Eastern Uplands	X	X		X	X	X	X	X		X
	Eastern Escarpment Mountains	X	X	X	X	X	X	X	X		X
	South Eastern Uplands	X	X	X	X	X	X	X	X	X	X
	North Eastern Coastal Belt	X	X	X	X	X	X	X	X		X
	Drought Corridor	X	X		X	X				X	X
	Southern Folded Mountains	X	X		X	X				X	X
	South Eastern Coastal Belt	X	X		X	X			X	X	X
	Great Karoo	X	X			X					X
	Southern Coastal Belt	X	X		X	X			X	X	X
	Western Folded Mountains	X	X		X	X			X		X
	South Western Coastal Belt	X	X		X	X				X	X
	Western Coastal Belt	X	X			X					X
	Nama Karoo	X	X		X	X			X		X
	Namaqua Highlands	NO SAMPLES IN THIS ECOREGION									
	Orange River Gorge	X	X		X	X	X				X
	Southen Kalahari	X	X		X	X	X		X		X
	Ghaap Plateau	X	X		X	X					X
	Eastern Coastal Belt	X	X	X	X	X			X	X	X

The Heptageniidae are known from all continents except Australia and South America. There are three recognised genera in the Afrotropics with only *Afronurus* and *Compsoneuriella* occurring in South Africa (Barber-James and Lugo-Ortiz 2003). Heptageniidae are found throughout South Africa where suitable habitat is available, although there are no records from the Lower Orange River downstream of the Namibian border (Figure 2.2 d). The Heptageniidae have a slightly broader distribution pattern than what is proposed by Picker *et al*. (2003), with the distribution extending to the Vaal-, Harts- and mid- to lower Orange Rivers. Heptageniidae were recorded in all the sampled ecoregions except the Northern Plateau at altitudes up to 3000 m a.m.s.l. (Table 2.1).

Leptophlebiidae have a worldwide distribution with the highest diversity in the tropics (Barber-James and Lugo-Ortiz 2003). Although seven genera have been recorded in South Africa, two of these genera are only known from adults (Barber-James and Lugo-Ortiz 2003). The Leptophlebiidae occur throughout South Africa with the exception of the very dry areas in the Northern Cape and Northwest Provinces (Figure 2.2 e). The Leptophlebiidae have been found in all the Level I Ecoregions except for the Namaqua Highlands (no information), the Natal Coastal Plain, the Ghaap Plateau and the Northern Plateau. Leptophlebiidae occurred in all the geomorphological zones and at altitudes up to 3500 m a.m.s.l. (Table 2.1). This distribution differs considerably from that suggested by Picker *et al.* (2003). According to Picker *et al.* (2003), the Leptophlebiidae only occur in a relatively narrow band from the eastern part of Swaziland, through the eastern sections of KZN, the eastern and southern Cape to more-or-less the mouth of the Olifants River on the west coast.

The Oligoneuriidae are known from all continents except Australia. Both Afrotropical genera (*Elassoneuria* and *Oligoneuriopsis*) occur in South Africa. Oligoneuridae are found in fast flowing streams, mainly at high elevations (Barber-James and Lugo-Ortiz 2003) although they have also been found at lower altitudes (Figure 2.2 f and Table 2.1). According to Agnew (1985), *Elassoneuria* is a tropical genus found in large, warm rivers, whereas *Oligoneuriopsis* is confined to high-lying streams in the Drakensberg escarpment. Their preference for very fast flowing water makes it difficult to collect these nymphs as most of the sampling occurs during the low-flow season. It is therefore likely that the distribution shown in Figure 2.2 f might be broader. Oligoneuridae are found mostly in the northern regions of South Africa, as well as along the eastern part of South Africa in KZN and the Eastern Cape (Figure 2.2 f).

The Polymitarcyidae is a pan-tropical family absent from Australia with three genera found in South Africa one of which is restricted to KZN (Barber-James and Lugo Ortiz 2003). Table 2.1 and Figure 2.2 g indicate the distribution of Polymitarcyidae in South Africa. The

Polymitarcyidae are restricted to the northern part of South Africa with an extension into southern KZN and the northern part of the Eastern Cape (figure 2.2 g). They occur in all geomorphological zones at altitudes up to 2500 m a.m.s.l. (Table 2.1). The distribution in Figure 2.2 g indicates a broader distribution than what is shown in Picker *et al.* (2003).

The Prosopistomatidae are known from only one genus *Prosopistoma* which is found in the Afro-tropics, Australia, the Orient and Europe (Barber-James and Lugo-Ortiz 2003). A recent study by Barber-James (2010) indicates that three species occur in South Africa. These tiny nymphs are easily overlooked in a SASS tray and might actually have a wider distribution than indicated in Table 2.1 and Figure 2.2 h with Picker *et al.* (2003), possibly reflecting a more accurate distribution of the family. However, the distribution in Figure 2.2 h also reflects the distribution map provided in Barber-James (2010). According to Agnew (1985) this family does not occur in the southern and western Cape. This is also evident in the distribution map presented in Picker *et al*. (2003), but from Figure 2.2 h it can be seen that prosopistomatids have been recorded in the southern- (Moordkuil and Keurbooms Rivers) and western (Olifants River) Cape. This family is found in the upper reaches of the Vaal River catchment as well as in the Harts- and Orange Rivers (Albany Museum Grahamstown 2014). The Prosopistomatidae occur in all geomorphological zones at altitudes up to 2500 m a.m.s.l (Table 2.1).

The Teloganodidae family is primarily found in the southern and south-western region of the Cape (Barber-James and Lugo-Ortiz 2003) with the northern limit of this family in the Hogsback area in the Eastern Cape (Barber-James and Pereira-da-Conceicoa pers. com. 2016). Recent work by Barber-James and Pereira-da-Conceicoa (pers. com.2016) found three more genera and ten new species of Teloganodidae in South Africa. The teloganodid species have restricted distribution with only limited overlap between the genera and species distribution (Barber-James and Pereira-da-Conceicoa pers. Com. 2016). Although there are some records further north, these are probably misidentification of the baetid mayflies *Acanthiops varius* or *A. tsitsa* (Barber-James pers. comm. 2014). These more northern records from the Freshwater Invertebrate Collection (Albany Museum Grahamstown 2014) and the rivers database have been excluded in Figure 2.2 i and Table 2.1. The Teloganodidae occur in all geomorphological zones at altitudes up to 1000 m a.m.s.l. (Table 2.1).

According to Barber-James and Lugo-Ortiz (2003) two (*Dicercomyzon* and *Tricorythus*) of the seven recognised Afro-tropical Tricorythidae genera occur in South Africa. However, since then the genus *Dicercomyzon* has been removed from the Tricorythidae and placed in the Dicercomyzidae family (Jacobus and McCafferty 2006). The information used in this chapter includes *Dicercomyzon* within the Tricorythidae because the data is mostly captured at family level and it is therefore not possible to exclude the *Dicercomyzon* from the records. There are five localities in the Freshwater invertebrate collection (Albany Museum Grahamstown 2014) where *Dicercomyzon* has been found previously. Three of these localities are in the Lowveld Ecoregion at altitudes between 150 and 350 m a.m.s.l, one in the Waterberg at an altitude of 1140 m a.m.s.l. and one in the Eastern Bankenveld at an altitude of 1170 m a.m.s.l. These five localities all occur within the lower foothills geomorphological zone. These five *Dicercomyzon* localities have been excluded from the distribution records. Tricorythidae have a very widespread distribution in South Africa (Figure 2.2 j, Table 2.1) with only the dry areas of the Karoo, Northern Cape and Limpopo provinces without distribution records. These areas are characterised by non-perennial streams and only limited surveys have been conducted in ephemeral and episodic rivers. The distribution records in Figure 2.2 j differ considerably from that in Picker *et al*. (2003) with the main difference being that Tricorythidae have been recorded at numerous sites in the southern and western Cape while this whole region has been excluded in Picker *et al.* (2003).

2.3.2 Trichoptera

Trichoptera is distributed throughout the world except for certain oceanic islands and the Polar Regions (Morse 2014). There are three suborders and 19 families of Trichoptera in Southern Africa (de Moor and Scott 2003). The Hydrosalpingidae and Barbarochthonidae are endemic to South Africa, but the Petrothrincidae which was also regarded as endemic to South Africa have now also been recorded from Madagascar (de Moor and Scott 2003). Recent studies (de Moor and Scott 2003), have shown that 10 of the 54 genera recorded in the Afrotropical region are endemic to South Africa. De Moor (1992) and de Moor and Scott (2003) described the geographical distribution of the South African trichopteran families according to the 12 Hydrobiological regions (Harrison 1959). The distribution of the trichopteran families per level I Ecoregion and geomorphological zones as well as the altitude ranges are indicated in Table 2.2.

Three species of a single genus (*Dipseupopsis*) of the Dipseudopsidae family have been recorded in the southern Cape as well as the eastern and northern regions of South Africa (de Moor and Scott 2003). The distribution according to de Moor and Scott (2003) is similar to what is presented in Picker *et al*. (2003), except that according to Picker *et al.* (2003) the family's distribution also includes portions of the Highveld and Central Arid Region whereas the Drakensberg and Middleveld regions are excluded by de Moor and Scott (2003). The distribution records (Figure 2.3 a) mostly coincide with the descriptions by de Moor and Scott (2003) as well as the distributions indicated in Picker *et al*. (2003). The main difference is that no records have been found in the Central Arid Region and thus the section along the border of Namibia is excluded from the distribution range. Dipseudopsidae occur in all geomorphological zones except the source zone and high-gradient mountain streams at altitudes up to 2000 m a.m.s.l. (Table 2.2).

Three genera of Ecnomidae have been recorded in South Africa with one genus occurring throughout South Africa except for the north-western region, while the other two genera have a more limited distribution (de Moor and Scott 2003). However, the family level information from the Rivers Database and this study (Figure 2.3 b and Table 2.2) indicates that Ecnomidae do occur in the north-western region excluded by de Moor and Scott (2003). According to Picker *et al*. (2003), this family occurs throughout the whole of South Africa and it would therefore not be unreasonable to extend the distribution range in Figure 2.3 b to also include the dry areas where there are no records in the sources used for this study. Ecnomidae occur in all sampled Ecoregions (except the Ghaap plateau), geomorphological zones (except the source zone) and at altitudes up to 3500 m.a.m.s.l. (Table 2.2).

The Hydropsychidae is a diverse and widespread family with eight genera occurring in South Africa (de Moor and Scott 2003). This family can be found throughout the whole of South Africa (de Moor 1992, de Moor and Scott 2003, Picker *et al*. 2003) as also indicated in Table 2.2. The recorded distribution of the Hydropsychidae family (Figure 2.3 c) corresponds well with the range of sites used in this study (Figure 2.1). This distribution range (Figure 2.3 c) also corresponds to the previously described ranges (de Moor 1992, Picker *et al*. 2003, de Moor and Scott 2003) except that the Kalahari Desert region on the border of Namibia and Botswana has been excluded. Hydropsychidae have been recorded in all the sampled ecoregions and geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.2).

Philopotamidae are represented by only two genera in southern Africa occurring from the south-western Cape along the eastern part of South Africa to the Mpumalanga and Limpopo Lowveld regions (de Moor and Scott 2003). According to Picker *et al.* (2003) the distribution extends to the north-western part of Limpopo as well. The distribution indicated in Figure 2.3 d, expands the range of the Philopotamidae to include most of the north eastern region of South Africa as well. The only regions where this family does not occur are the western parts of the North West Province as well as the northern sections of the Northern Cape (Figure 2.3 d). Philopotamidae have been recorded in all geomorphological zones (except the source zone) at altitudes up to 2500 m a.m.s.l (Table 2.2).

Polycentropodidae are represented by five genera in southern Africa of which only two occur in South Africa (de Moor and Scott 2003). According to de Moor and Scott (2003), Polycentropodidae occur throughout South Africa except for northern KZN, Mpumalanga, Limpopo and the northern portions of the North West Province. The distribution indicated in Figure 2.3 e and Table 2.2 extends to include these northern areas of South Africa as well. Polycentropodidae have been recorded from all geomorphological zones, except the source zone, at altitudes of up to 2000 m a.m.s.l. (Table 2.2).

No distinction is made between Psychomyiidae and Xiphocentronidae in the SASS system due to the difficulties in distinguishing between the two families in the field. Two psychomyiid genera and a single xiphocentronid genus occur in South Africa (de Moor and Scott 2003). According to de Moor and Scott (2003) these two families occur in a band from the Olifants River on the west coast (the Cape System region) along the southern- and eastern- Cape to the vicinity of St. Lucia (the South-East Coastal Region) in northern KZN. The data sources used during this study indicate a broader distribution that extends inland and northwards to the borders with Zimbabwe and Mozambique (Figure 2.3 f and Table 2.2). These two families have been recorded from all geomorphological zones (except the source zone) and at altitudes up to 3500 m a.m.s.l. (Table 2.2).

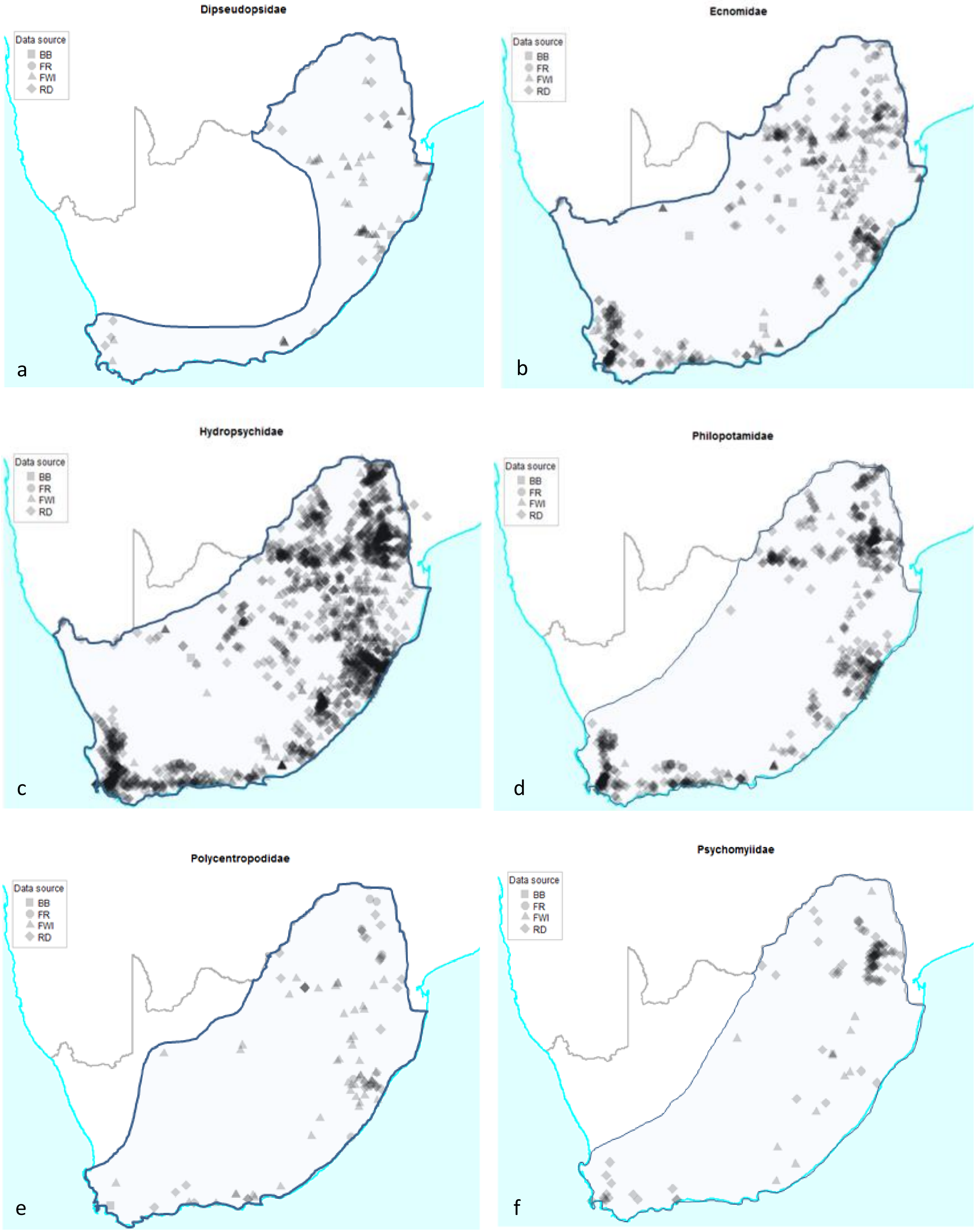

Figure 2.4: Distribution map of Trichoptera in South Africa: (a) Dipseudopsidae, (b) Ecnomidae, (c) Hydropsychidae, (d) Philopotamidae, (e) Polycentropodidae, (f) Psychomyiidae/Xiphocentronidae, (g) Barbarochthonidae, (h) Calamoceratidae, (i) Glossosomatidae, (j)Hydroptilidae, (k) Hydrosalpingidae, (l) Lepidostomatidae, (m) Leptoceridae, (n) Petrothrincidae, (o) Pisuliidae, (p) Sericostomatidae

According to de Moor and Scott (2003), the endemic Barbarochthonidae are represented by a single species, *Barbarochthon brunneum* in South Africa restricted to the Cape System region and the South-East Coastal Region. According to Scott (1985) barbarochthonids occur mainly in the western- Cape but have also been recorded in the southern- Cape and KZN. These descriptions also coincide with the distribution pattern displayed in Figure 2.3 g and Table 2.2, but according to de Moor (pers. comm. 2014) the distribution records in KZN are dubious as the family has only been confirmed from Hydrobiological region A (Cape System Region). The KZN records are from the Rivers Database and might be misidentifications or incorrect data-entry and have therefore been excluded from the distribution map. From Table 2.2 it can be seen that Barbarochthonidae have been recorded from all geomorphological zones, except for the source zone at altitudes up to 1000 m a.m.s.l.

The subtropical Calamoceratidae (Scott 1985) are represented in South Africa by a single species (*Anisocentrus usambarensis*) that is restricted to the South-Eastern Coastal region according to de Moor and Scott (2003) but has also been found in the north-eastern region of South Africa (Figure 2.3 h and Table 2.2). The distribution of the Calamoceratidae coincides with forested areas where they use fallen leaves to construct their cases (Scott 1985). The single record (from the Rivers Database) for this family in the Western Cape is clearly incorrect (Barber-James pers. comm. 2014) and was excluded from the distribution map. This record might be an incorrect entry or a misidentification. Table 2.2 indicates that this family has been recorded in the transitional zone, foothills and lowland river geomorphological zones at altitudes up to 1500 m a.m.s.l.

The Glossosomatidae are represented by two species in a single genus (*Agapetus* (*Synagapetus*)) that is restricted to the Cape System and South-East Coastal regions in South Africa (de Moor and Scott 2003). According to Scott (1985) this family may be locally common in suitable localities in the southern- and western Cape. The distribution range in Picker *et al.* (2003) is similar to that described by de Moor (1992) and de Moor and Scott (2003) but it also includes sections of the Eastern Cape and Drakensberg Mountain regions. Figure 2.3 i and Table 2.2 indicate a similar distribution as for Barbarochthonidae with the majority of locations in the southern- and western cape (Cape System) but with two localities (from the rivers database) in the South-East Coastal region in KZN. According to de Moor (pers. comm. 2014) the Drakensberg records from the Rivers Database are incorrect and likely to be either misidentifications or incorrect data entries and these records have therefore been excluded from

Figure 2.3 i and Table2.2. Glossosomatidae have been recorded from all geomorphological zones except the source zone at altitudes up to 1000 m a.m.s.l. (Table 2.2).

The Hydroptilidae are widespread in South Africa and are represented by five genera as well as an undescribed genus in the tribe Leucotrichinii that has been found in the Western Cape (de Moor and Scott 2003). There is evidence that two other genera (*Stenoxyerthira* and *Maydenoptila*) might also be found in Southern Africa (de Moor and Scott 2003) which would mean that there would possibly be eight hydroptilid genera in South Africa. The current distribution records used in this study (Figure 2.3 j and Table 2.2) indicate that this family actually also occur in the Middleveld region excluded by de Moor (1992) and de Moor and Scott (2003). Hydroptilidae havev been recorded from all sampled ecoregions (except the Northern Plateau and the Natal Coastal Plain) and geomorphological zones (except the source zone) at altitudes up to 3500 m a.m.s.l. (Table 2.2).

The Hydrosalpingidae are represented by a single species (*Hydrosalpinx sericea*) occurring in the Cape System Region in South Africa (de Moor and Scott 2003). There is only a single record of this family, on the Keisie River near Montagu in the Western Cape, on the Rivers Database (Figure 2.3 k) and it was not found during the recent surveys to collect data for this study and there are also no records of it on the Biobase (Dallas *et al*. 1999). The distribution range in Picker *et al*. (2003) coincides with the Cape System Region of Harrison (1959). The few museum records for this family are restricted to the Cape System Hydrobiological Region and two sites in KZN (Figure 2.3 k and Table 2.2). According to de Moor (Pers. Comm. 2014), the two records in KZN are incorrect and the specimens should be re-examined to clarify the identification. Scott (1985) described this family as a south-western Cape endemic that is rarely found due possibly to predation by trout. The Hydrosalpingidae have only been recorded from the transitional and upper foothills zones and is restricted to altitudes up to 1500 m a.m.s.l. (Table 2.2).

The Lepidostomatidae are represented by a single species (*Goerodes caffrariae*) in South Africa where it has been recorded in the Eastern Cape, South-East Coastal, Drakensberg Mountain and the Eastern Escarpment Hydrobiological regions according to de Moor and Scott (2003) but have also been found to have a more widespread distribution according to data from the sources used in this study (Figure 2.3 l and Table 2.2). This family has been recorded in all geomorphological zones (except the source zone) at altitudes up to 3000 m a.m.s.l. (Table 2.2).

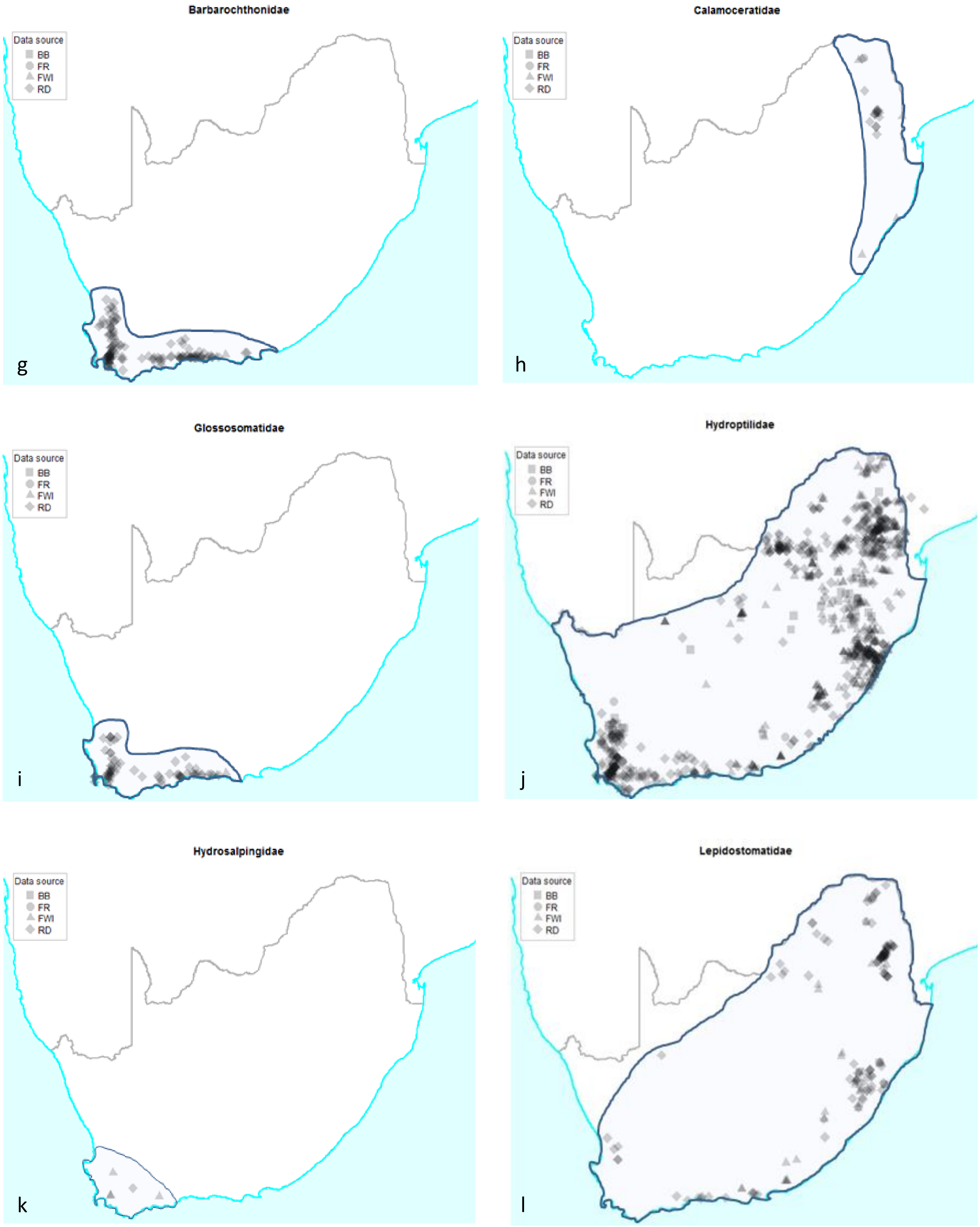

Figure 2.3 (continued): Distribution map of Trichoptera in South Africa: (a) Dipseudopsidae, (b) Ecnomidae, (c) Hydropsychidae, (d) Philopotamidae, (e) Polycentropodidae, (f) Psychomyiidae/Xiphocentronidae, (g) Barbarochthonidae, (h) Calamoceratidae, (i) Glossosomatidae, (j) Hydroptilidae, (k) Hydrosalpingidae, (l) Lepidostomatidae, (m) Leptoceridae, (n) Petrothrincidae, (o) Pisuliidae, (p) Sericostomatidae

Table 2.2: The distribution ranges of Trichoptera families based on distribution records obtained from the Biobase, Rivers Database, Freshwater Invertebrate Collection and a study to determine the environmental requirements of four different Insect orders.

	Trichoptera family	Dipseudopsidae	Ecnomidae	Hydropsychidae	Philopotamidae	Polycentropodidae	Psychomyiidae	Barbarochthonidae	Calamoceratidae	Glossosomatidae	Hydroptilidae	Hydropsalpingidae	Lepidostomatidae	Leptoceridae	Petrothrincidae	Pisuliidae	Sericostomatidae
	Altitude Range (m a.m.s.l.)	0-2000	0-3500	0-3500	0-2500	0-2000	0-3500	0-1000	0-1500	0-1000	0-3000	0-1500	0-3000	0-3500	0-1000	0-2000	0-2000
ZONE	Source zone			X										X			
	High-gradient Mountain Stream		X	X	X	X	X	X		X	X		X	X	X	X	X
	Mountain Stream	X	X	X	X	X	X	X		X	X		X	X	X	X	X
	Transitional Zone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Upper Foothills	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Lower Foothills	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
	Lowland	X	X	X	X	X	X		X		X		X	X		X	X
LEVEL I ECOREGION	Limpopo Plain		X	X	X		X				X			X			
	Soutpansberg		X	X	X		X		X		X		X	X			
	Lowveld	X	X	X	X	X	X		X		X		X	X			
	North Eastern Highlands	X	X	X	X	X	X		X		X		X	X		X	
	Northern Plateau		X	X													
	Waterberg		X	X	X		X				X		X	X			
	Western Bankenveld	X	X	X	X	X	X				X		X	X		X	
	Bushveld Basin		X	X	X						X			X			
	Eastern Bankenveld	X	X	X	X	X	X				X		X	X			
	Northern Escarpment Mountains		X	X	X	X	X		X		X		X	X		X	
	Highveld	X	X	X	X	X	X				X		X	X		X	
	Lebombo Uplands	X	X	X	X				X		X			X			
	Natal Coastal Plain	X	X	X					X					X			
	North Eastern Uplands	X	X		X	X					X			X		X	X
	Eastern Escarpment Mountains	X	X	X	X	X	X				X		X	X		X	X
	South Eastern Uplands	X	X	X	X	X	X	X	X		X		X	X		X	X
	North Eastern Coastal Belt	X	X	X	X	X	X	X			X		X	X		X	X
	Drought Corridor	X	X	X	X		X				X			X		X	
	Southern Folded Mountains	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
	South Eastern Coastal Belt		X	X	X	X		X		X	X		X	X	X	X	X
	Great Karoo		X	X	X						X			X			
	Southern Coastal Belt		X	X	X	X	X	X		X	X	X	X	X	X	X	X
	Western Folded Mountains	X	X	X	X	X		X		X	X	X	X	X	X	X	X
	South Western Coastal Belt	X	X	X	X		X	X		X	X			X	X	X	X
	Western Coastal Belt		X	X	X						X		X	X			X
	Nama Karoo		X	X		X					X		X	X			
	Namaqua Highlands	NO SAMPLES IN THIS ECOREGION															
	Orange River Gorge		X	X							X			X			
	Southen Kalahari		X	X	X	X	X				X		X	X		X	
	Ghaap Plateau			X	X						X		X	X			
	Eastern Coastal Belt			X	X						X			X		X	

The Leptoceridae is a very diverse and widespread family occurring throughout South Africa apart from the Tropical Arid Region (de Moor 1992, de Moor and Scott 2003). There are currently twelve genera recorded from South Africa as well as an unnamed genus and species collected by de Moor in 1997 from the Cape System Region (de Moor and Scott 2003). The data sources used in this study also includes records for the Tropical Arid Region corresponding to the distribution range suggested by Picker *et al*. (2003). This family occurs throughout the whole of South Africa apart from the Kalahari Desert on the border with Namibia and Botswana (Figure 2.3 m). Leptoceridae have been recorded from all geomorphological zones and at altitudes up to 3500 m a.m.s.l. (Table 2.2).

The Petrothrincidae are represented by a single genus (*Petrothincus*) with three species in South Africa and the distribution limited to the Cape System and the South-East Coastal regions (de Moor 1992, de Moor and Scott 2003). Only one (*P. circularis*) of the three species occurs in the South-East Coastal Region (de Moor and Scott 2003). According to Scott (1985) this genus is endemic to the south-western Cape. Petrothrincidae only occur in the south-western Cape (Figure 2.3 n) and not in the Eastern Cape, Drakensberg Mountain or the South-East Coastal regions as indicated previously (de Moor 1992, Picker *et al*. 2003, de Moor and Scott 2003). This discrepancy in the distribution records is unclear as more information was used during the current study than was used previously. The limited records from the Rivers Database might be partly explained by the cryptic nature of this family making it difficult to see in a SASS tray. Petrothrincidae have been recorded in only four ecoregions and in all geomorphological zones except for the source zone and lowland rivers at altitudes up to 1000 m a.m.s.l. (Table 2.2).

The Pisuliidae are represented by two genera in South Africa (de Moor and Scott 2003) occurring in the Cape System, the Eastern Cape, the South-East Coastal, and the Transvaal Mountain regions (de Moor 1992, de Moor and Scott 2003). Picker *et al.* (2003) expands the distribution range to also include the southern sections of the Lowveld Hydrobiological region. A similar distribution range is indicated in Figure 2.3 o; however the range is extended to also include parts of the Central Arid, Tropical Arid and Middleveld regions. Pisuliidae have been recorded in all geomorphological zones (except the source zone) at altitudes up to 2000 m a.m.s.l. (Table 2.2).

The sericostomatid caddisflies are represented by five southern African genera endemic to South Africa (Scott 1985, de Moor and Scott 2003). This family is mostly restricted to the Cape System Region except for one genus (*Aclosma*) that occurs in the Eastern Cape, the Drakensberg Mountain- and the South-East Coastal regions (de Moor 1992, de Moor and Scott 2003). Picker *et al*. (2003) expands the range to also include the southern sections of the Lowveld but indicates a narrower distribution range restricted mostly to the coastal regions. The distribution range in Figure 2.3 p and Table 2.2 coincides to a large degree with these ranges but excludes the northern distributions into the Lowveld regions (de Moor 1992, de Moor and Scott 2003, Picker *et al.* 2003). This family has been recorded from all geomorphological zones (except the source zone) and at altitudes up to 2000 m a.m.s.l. (Table 2.2).

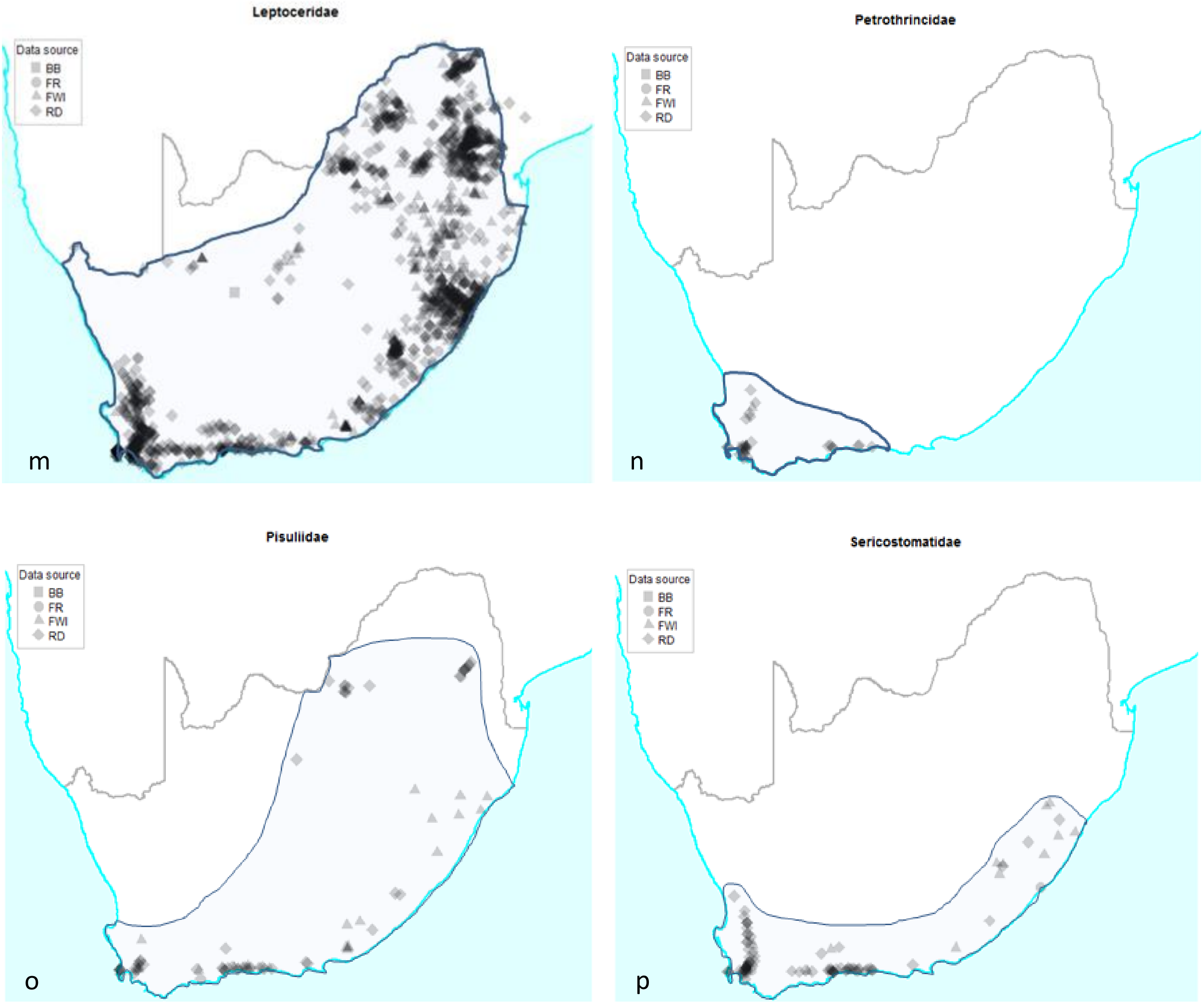

Figure 2.3 (continued): Distribution map of Trichoptera in South Africa: (a) Dipseudopsidae, (b) Ecnomidae, (c) Hydropsychidae, (d) Philopotamidae, (e) Polycentropodidae, (f) Psychomyiidae/Xiphocentronidae, (g) Barbarochthonidae, (h) Calamoceratidae, (i) Glossosomatidae, (j) Hydroptilidae, (k) Hydrosalpingidae, (l) Lepidostomatidae, (m) Leptoceridae, (n) Petrothrincidae, (o) Pisuliidae, (p) Sericostomatidae

2.3.3 Coleoptera

The Coleoptera are probably the largest order of living organisms with approximately 370 000 described species although the estimates of total number of extant species are estimated to be between one and five million (Gaston 1991). The coleopterans differ from many other aquatic insects in the sense that many of the aquatic beetles are aquatic in both the larval and the aquatic stages. Jäch (1998) developed a functional/ecological classification system for the so-called "water beetles" in which he distinguished between true, false, phytophilous, parasitic and facultative water beetles with shore beetles as the sixth group. The beetles in the SASS system include only the true water beetles, the false water beetles and the shore beetles.

True water Beetles (Gyrinidae, Haliplidae, Dytiscidae, Noteridae, Hydrophilidae, Hydraenidae, Elmidae and Dryopidae) have the adult stage submerged most of the time while the larvae and pupae might be aquatic or terrestrial. The false water beetles (Scirtidae, Psephenidae and Ptilodactylidae) have aquatic larvae while the adults are terrestrial. Shore beetles (Limnichidae) are, strictly speaking, terrestrial, but in all their developmental stages are found almost exclusively very close to the water's edge or in very wet habitats.

In the SASS system the Noteridae (burrowing water beetles) are included with the Dytiscidae (predacious diving water beetles) because it is difficult to distinguish between them in the field and they were previously considered as a subfamily of the Dytiscidae (Endrödy-Younga and Stals 2008a). Only four of the 14 noterid genera are found in southern Africa. Dytiscids are true water beetles with both adults and larvae being aquatic. The Dytiscidae are also the largest of all aquatic beetle families and are found worldwide (Biström 2008). Dytiscidae may also occur in temporary water bodies because many species are able to fly (Biström 2008). Thirty eight genera have been recorded in South Africa and the family is widespread through the country (Stals 2008a). According to Stals (2008a), Dytiscidae have been recorded from all nine provinces in South Africa whereas the Noteridae are restricted to the Eastern Cape, KZN, Mpumalanga and Limpopo. Picker *et al*. (2003) indicate that the Dytiscidae occur throughout the whole of South Africa that also coincides with the results from this study (Figure 2.4 a). These two families have been recorded from all sampled ecoregions (except the Northern Plateau), from all geomorphological zones and at altitudes up to 3000 m a.m.s.l. (Table 2.3).

In the SASS system the Dryopidae (long toed water beetles) are included with the Elmidae (riffle beetles) because both families are very small and are difficult to distinguish in the field. In earlier classifications the Dryopidae were often lumped with the Elmidae and the Lutrochidae (Nelson 2008b). Both families are found on all continents except Antarctica (Nelson 2008a, Nelson 2008b). Elmidae are true water beetles that are aquatic in all stages, while the non-aquatic dryopid larvae are found in leaf litter or soil near the water (Nelson 2008b). Twenty genera of Elmidae (17 in South Africa) and three genera of Dryopidae have been recorded in southern Africa although it is likely that there are many undescribed species (Nelson 2008a, Nelson 2008b). It is also suspected that the genus, *Dryops*, occurs in the region although it has not yet been recorded (Nelson 2008b). The Elmidae occur widely throughout South Africa wherever there is suitable habitat. Only one of the dryopid genera occurs widely in South Africa while the other two genera are restricted to the Western- and Eastern Cape provinces (Stals 2008a). According to Stals (2008a) the Elmidae have not been recorded from the Free State and North West provinces although there are records from the Vaal River. As can be seen from Figure 2.4 b and Table 2.3 these two families occur throughout the whole of South Africa apart from the very dry Kalahari Desert region on the border with Namibia and Botswana. Their distribution in the very dry areas of the Northern Cape is likely to be restricted to the few perennial rivers such as the Orange- and Vaal rivers. These two families have been recorded from all sampled ecoregions (except the Natal Coastal Plain) and geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.3).

The Gyrinidae (whirligig beetles) are a cosmopolitan family of true water beetles with both adults and larvae being strictly aquatic (Stals 2008b). There are four gyrinid genera in South Africa and they have been recorded from all nine provinces (Stals 2008a). According to Stals (2008b), gyrinids are frequently found in the southern-, eastern- and northern parts of southern Africa where there is permanent water but much less frequently in the dry western section and the Central Plateau, although some species can be found in temporary water bodies. The distribution range for the Gyrinidae presented in Figure 2.4 c and Table 2.3 span the whole of South Africa as also indicated in Picker *et al.* (2003) but the Kalahari Desert on the border with Namibia and Botswana is excluded. The Gyrinidae have been recorded from all sampled ecoregions (except for the Northern Plateau) and geomorphological zones at altitudes up to 3000 m a.m.s.l. (Table 2.3).

The Haliplidae (crawling water beetles) are true water beetles that are strictly aquatic in both adult and larval stages (Endrödy-Younga and Stals 2008b). There are presently three haliplid genera known from South Africa (Biström 1985, Stals 2008a). According to Stals (2008a), Haliplidae occur in all provinces except the Limpopo and North West Provinces. Haliplids are not commonly found during biomonitoring surveys but are reasonably widespread throughout South Africa (Figure 2.4 d and Table 2.3) except for the dry north-western region of the Northern Cape and North West Province. Haliplidae have been recorded from all geomorphological zones, except for the source zone at altitudes up to 2000 m a.m.s.l. (Table 2.3).

The Scirtidae (marsh beetles) are false water beetles with the adults invariably terrestrial while the larvae are usually aquatic (Endrödy-Younga and Stals 2008c). Although this family is recorded as Helodidae on the Rivers Database and the SASS system, the correct name is Scirtidae and must be corrected on both the SASS5 field forms and the Rivers Database. Very little is known about the Scirtidae of southern Africa and no checklist is available (Endrödy-Younga and Stals 2008c). This family occurs reasonably widely throughout South Africa but has not yet been recorded in the Karoo and Northern Cape areas (Figure 2.4 e). The Scirtidae have been recorded from all geomorphological zones, except for the source zone, and at altitudes up to 3500 m a.m.s.l. (Table 2.3).

The Hydraenidae (minute moss beetles) have a cosmopolitan distribution. Although the southern African hydraenids do not have many species, it is a very diverse and highly endemic family (Perkins 2008). According to Perkins (2008), this family includes truly aquatic, semi-aquatic and humicolous taxa. There are 13 aquatic or semi-aquatic hydraenid genera in South Africa (Stals 2008a) with a widespread distribution occurring in all nine provinces. The Hydraenidae occur throughout the major part of South Africa with only the very dry desert and semi-desert areas excluded (Figure 2.4 f). The Scirtidae have been recorded from all the geomorphological zones, and at altitudes up to 3500 m a.m.s.l. (Table 2.3). They have not yet been recorded from the Northern Plateau or the Natal Coastal Plain (Table 2.3).

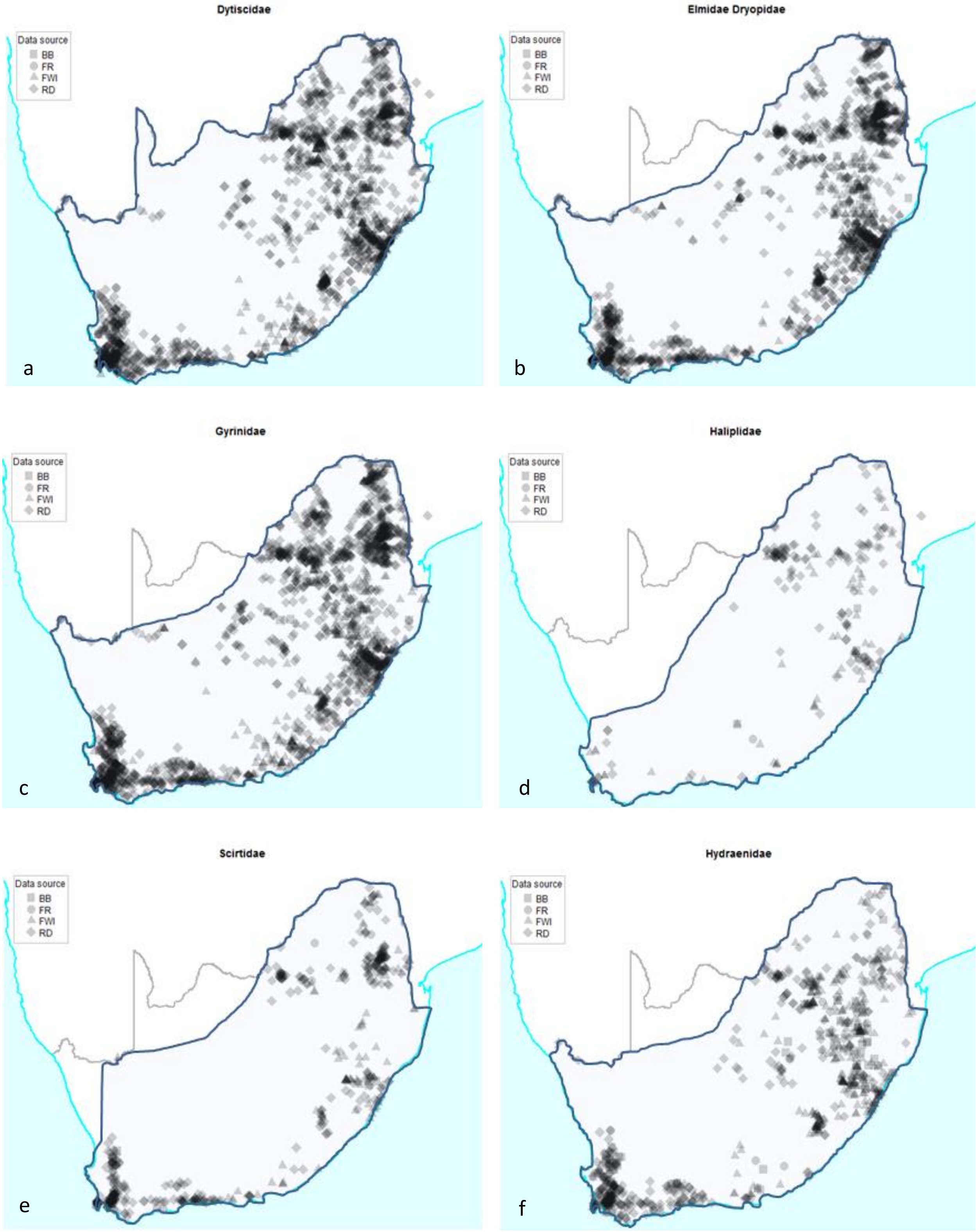

Figure 2.5: Distribution map of aquatic Coleoptera in South Africa: (a) Dytiscidae/Noteridae, (b) Elmidae/Dryopidae, (c) Gyrinidae, (d) Haliplidae, (e) Scirtidae, (f) Hydraenidae, (g) Hydrophilidae, (h) Limnichidae, (i) Psephenidae, (j) Ptilodactylidae

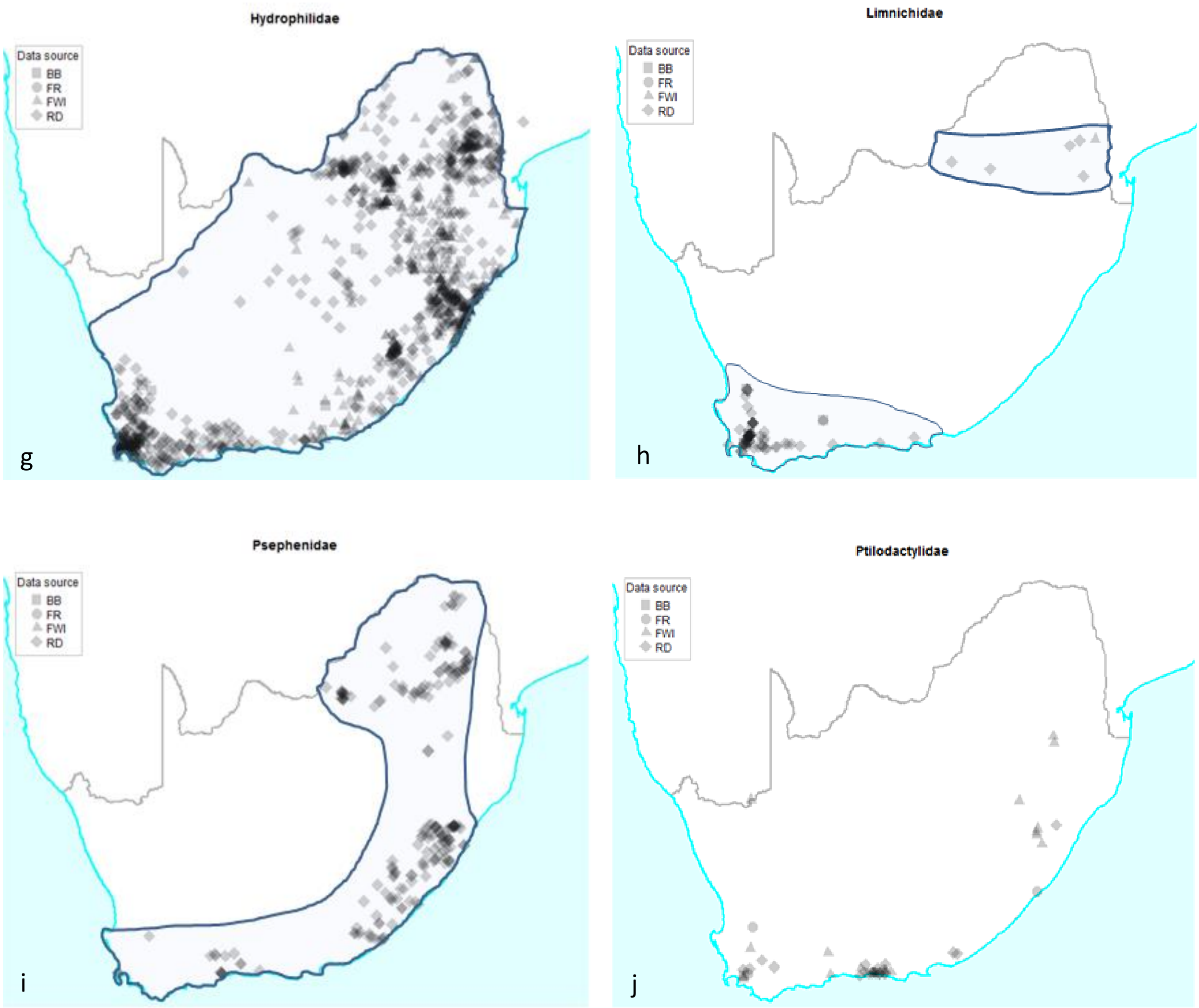

Figure 2.6 (continued): Distribution map of aquatic Coleoptera in South Africa: (a) Dytiscidae/Noteridae, (b) Elmidae/Dryopidae, (c) Gyrinidae, (d) Haliplidae, (e) Scirtidae, (f) Hydraenidae, (g) Hydrophilidae, (h) Limnichidae, (i) Psephenidae, (j) Ptilodactylidae

The Hydrophilidae (water scavenger beetles) belong to the water beetle family with the second highest number of species and is encountered in almost any freshwater habitat. Hydrophilids are true water beetles with both adult and larval stages living in water (Stals and Endrödy-Younga 2008c). Although the southern African hydrophilid fauna is reasonably well known, comprehensive revisions on the family only became available recently (Stals and Endrödy-Younga 2008c). There are 16 hydrophilid genera occurring right throughout South Africa in all nine provinces (Stals 2008a). The distribution range for this family is indicated in Figure 2.4 g and Table 2.3 with only the Kalahari Desert on the border of Namibia and Botswana excluded. The Hydrophilidae have been recorded from all sampled ecoregions (except the Northern Plateau and Orange River Gorge) and from all geomorphological zones (except the source zone) at altitudes up to 3000 m a.m.s.l. (Table 2.3).

Table 2.3: The distribution ranges of Coleoptera families based on distribution records obtained from the Biobase, Rivers Database, Freshwater Invertebrate Collection and a study to determine the environmental requirements of four different Insect orders.

	Coleoptera Family	Dytiscidae/Noteridae	Elmidae	Gyrinidae	Haliplidae	Scirtidae	Hydraenidae	Hydrophilidae	Limnichidae	Psephenidae	Ptilodactylidae
	Altitude Range (m a.m.s.l.)	0-3000	0-3500	0-3000	0-2000	0-3500	0-3500	0-3000	0-2000	0-2000	0-3000
ZONE	Source zone	X	X	X			X				
	High-gradient Mountain Stream	X	X	X	X	X	X	X	X	X	
	Mountain Stream	X	X	X	X	X	X	X	X	X	X
	Transitional Zone	X	X	X	X	X	X	X	X	X	X
	Upper Foothills	X	X	X	X	X	X	X	X	X	X
	Lower Foothills	X	X	X	X	X	X	X	X	X	X
	Lowland	X	X	X	X	X	X	X	X	X	X
LEVEL I ECOREGION	Limpopo Plain	X	X	X	X	X	X				
	Soutpansberg	X	X	X	X	X	X			X	
	Lowveld	X	X	X	X	X	X	X	X	X	
	North Eastern Highlands	X	X	X	X	X	X	X	X	X	
	Northern Plateau	X	X	X		X					
	Waterberg	X	X	X	X		X		X	X	
	Western Bankenveld	X	X	X	X	X	X	X	X	X	X
	Bushveld Basin	X	X	X	X	X	X		X	X	
	Eastern Bankenveld	X	X	X	X	X	X		X	X	
	Northern Escarpment Mountains	X	X	X	X	X	X	X	X	X	
	Highveld	X	X	X	X	X	X		X	X	X
	Lebombo Uplands	X	X	X		X	X		X		
	Natal Coastal Plain	X		X	X				X		
	North Eastern Uplands	X	X	X	X	X	X		X	X	
	Eastern Escarpment Mountains	X	X	X	X	X	X		X	X	X
	South Eastern Uplands	X	X	X	X	X	X		X	X	X
	North Eastern Coastal Belt	X	X	X	X	X	X		X	X	
	Drought Corridor	X	X	X	X	X	X		X	X	
	Southern Folded Mountains	X	X	X	X	X	X	X	X	X	X
	South Eastern Coastal Belt	X	X	X	X	X	X	X	X	X	X
	Great Karoo	X	X	X			X		X		
	Southern Coastal Belt	X	X	X		X	X	X	X	X	X
	Western Folded Mountains	X	X	X	X	X	X	X	X	X	X
	South Western Coastal Belt	X	X	X	X	X	X	X	X	X	X
	Western Coastal Belt	X	X	X			X		X		
	Nama Karoo	X	X	X			X		X		X
	Namaqua Highlands	NO SAMPLES IN THIS ECOREGION									
	Orange River Gorge	X	X	X		X	X				X
	Southen Kalahari	X	X	X			X		X		
	Ghaap Plateau	X	X	X	X		X		X		
	Eastern Coastal Belt	X	X	X		X	X		X	X	

The Limnichidae (minute marsh-loving beetles) are shore beetles that are often associated with muddy or sandy banks. The larvae are terrestrial and pupation occurs in the larval habitat while the adults are most often found in shore-line debris (Endrödy-Younga 2008). According to Endrödy-Younga (2008) the apparent scarcity of Limnichidae in southern Africa is most likely a reflection of inadequate or inappropriate collecting techniques. Only one southern African species (*Afrolimnichus oblongus*) has been described and identified, but other taxa are also known from the region (Penrith 1985a, Endrödy-Younga 2008). Museum records and data from the Rivers Database (Figure 2.4 h and Table 2.3) indicates that the limnichids have been found mostly in the Western Cape with a few records further north in North West, Limpopo and Mpumalanga provinces. The Limnichidae has been recorded from all geomorphological zones, except the source zone, at altitudes up to 2000 m a.m.s.l. (Table 2.3).

The Psephenidae (water-penny beetles) occur on all continents except Antarctica, with the highest diversity in the northern hemisphere. Only 13 species in three genera have been described in sub-Saharan Africa but many undescribed taxa are known to specialists (Shepard and Lee 2008). All psephenids are aquatic as larvae and riparian as adults and are therefore classified as false water beetles. According to Shepard and Lee (2008) the knowledge of these beetles are so limited that it would be premature to evaluate possible endemism. Stals (2008a) indicated that this family has been found in all nine provinces whereas Picker *et al*. (2003) indicated the distribution as being restricted to the northern and north-eastern part of South Africa. The three genera from South Africa have a reasonably widespread distribution throughout the country except for the extreme eastern parts of Limpopo, Mpumalanga and KZN, as well as the dry Karoo, Northern Cape and the western portion of the Western Cape (Figure 2.4 i and Table 2.3). Psephenidae have been recorded from all geomorphological zones, except the source zone, at altitudes up to 2000 m a.m.s.l (Table 2.3).

Although Ptilodactylidae (toe-winged beetles) are not included in the current SASS system, they occur relatively frequently in the Western- and Eastern Cape. It was decided to include the ptilodactids in this chapter as they might conceivably be included in future versions of SASS and it was relatively easy to obtain a record of the sites where they have been found during biomonitoring surveys. The Ptilodactylidae have an almost world-wide distribution, but are absent from Europe (de Moor 2008). Although the larvae of several ptilodactylid species are known to be aquatic, the adults seem to be terrestrial making this family false water beetles (de Moor 2008). Very little is known about the South African ptilodactylid fauna. Although there are some adult ptilodactylid specimens in some South African insect collections, they remain unidentified and possibly undescribed. In addition it is also not known which of these unidentified beetles might have aquatic larvae (de Moor 2008). Due to the uncertainty with regards to this family, no attempt has been made to draw a distribution range. The distribution of the ptilodactylid larvae as indicated in Figure 2.4 j and Table 2.3, although by no means comprehensive, is at least a start in getting an idea about the geographical range of the aquatic members of this family. This family has been found in all geomorphological zones except the source zone and high-gradient mountain streams at altitudes up to 3000 m a.m.s.l. (Table 2.3).

2.3.4 Diptera

All adult dipterans are terrestrial but the immature stages of many species are aquatic. Although some of the aquatic dipteran families have been thoroughly studied in South Africa, very little is known about many others (Harrison *et al*. 2003a). According to Harrison *et al*. (2003a), most dipteran genera with aquatic larvae are probably represented in southern Africa although there is uncertainty since some of the families have been very poorly studied. Most of aquatic dipteran families that occur in sub-Saharan Africa co-occur in all the major regions of the world and many of the genera have worldwide distribution (Harrison *et al.* 2003a). Although there are a few species common to Africa and Eurasia most of the species found in sub-Saharan Africa are endemic to Africa (Harrison *et al*. 2003a). The distribution of the Dipteran families per level I Ecoregion and geomorphological zones as well as the altitude ranges are indicated in Table 2.4.

According to Stuckenberg (1960), the South African Athericidae consists of only three genera, with the *Pachybates* and *Trichacantha* entirely endemic to the mountains of the western and southern Cape. The third genus (*Suragina*) has a worldwide distribution and is widespread in southern Africa with known records from the Free State, KZN, Mpumalanga, North West and Limpopo provinces within South Africa (Harrison *et al*. 2003b). Only the larvae of the Athericidae are aquatic as pupation occurs in the soil on river banks (Harrison *et al*. 2003b). Athericidae occur through most of South Africa apart from the very dry areas of the Northern Cape (Figure 2.5 a). Although the larvae are found in rivers, they have not been recorded from the lower Orange River. Athericidae have been recorded from all geomorphological zones, except the source zone, at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The Blephariceridae (net-winged midges) are a small family widely distributed through the world, with many species endemic to reasonably small areas. All blephariceridid larvae and pupae are aquatic (Harrison *et al*. 2003c). According to Stuckenberg (1980) there is only one blephariceridid genus (*Elporia*) in South Africa with 19 described species. According to Harrison *et al*. (2003c), the Blephariceridae have been recorded from the Cape Fold Mountains and the Great Escarpment and the Drakensberg Mountains from the Eastern Cape to the Limpopo province but Stuckenberg (1980) found no Blephariceridae in the Zoutpansberg. Picker *et al*. (2003) indicates the distribution from the Olifants River on the West Coast in a coastal band around the southern Cape before becoming broader in the Eastern Cape and KZN extending along the mountainous areas north and a thin strip extending southwest through Gauteng and the border between the Free State and the North West Provinces. Blephariceridae are quite sensitive and are only able to survive in cool, well-oxygenated, unpolluted mountain streams (Barraclough and Londt 1985). The distribution in Figure 2.5 b and Table 2.4 indicates two main distribution ranges in the south-western Cape and then along the mountainous areas of the Eastern Cape, KZN, Mpumalanga and Limpopo. Blephariceridae have also been found in the upper reaches of the Groot Marico- and Kaaloog se Loop Rivers in the North West province. The distribution is mainly restricted to mountainous regions where the water temperatures are expected to be lower. Blephariceridae have been recorded from all geomorphological zones (except the source zone) and at altitudes up to 3500 m a.m.s.l. (Table 2.4). Specimens have been collected from 120 localities ranging from the Groot River near Natures Valley at an altitude of 22 m a.m.s.l to the upper reaches of the Thukela River at an altitude of 3076 m a.m.s.l with a median altitude of 885 m a.m.s.l. The localities in the Western Cape tend to be lower than those in the rest of the country.

The Ceratopogonidae (biting midges) are a very large family of 4732 species in 89 genera worldwide occurring in nearly every aquatic or semi-aquatic habitat in all regions (de Meillon and Wirth 1991, de Meillon and Wirth 2003). According to de Meillon and Wirth (2003) there are fifty ceratopogonid genera in the Afrotropical region with fifteen genera endemic to the Afrotropics. The distribution in Figure 2.5 c and Table 2.4 coincides with that suggested by Picker *et al*. (2003). The Ceratopogonidae have been recorded in all sampled ecoregions except the Northern Plateau and in all geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.4).

According to Harrison (2003) the Chironomidae (non-biting midges) constitute at least 50% of the total number of macroinvertebrate species present in inland waters and can exploit any wet or damp habitat. Unlike many other aquatic insect taxa, the chironomid fauna of southern Africa, do not display much local endemism. The Chironomidae occur widely throughout the whole of South Africa (Figure 2.5 d) as was also suggested by Picker *et al*. (2003). The Chironomidae have been recorded in all sampled ecoregions and geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.4).

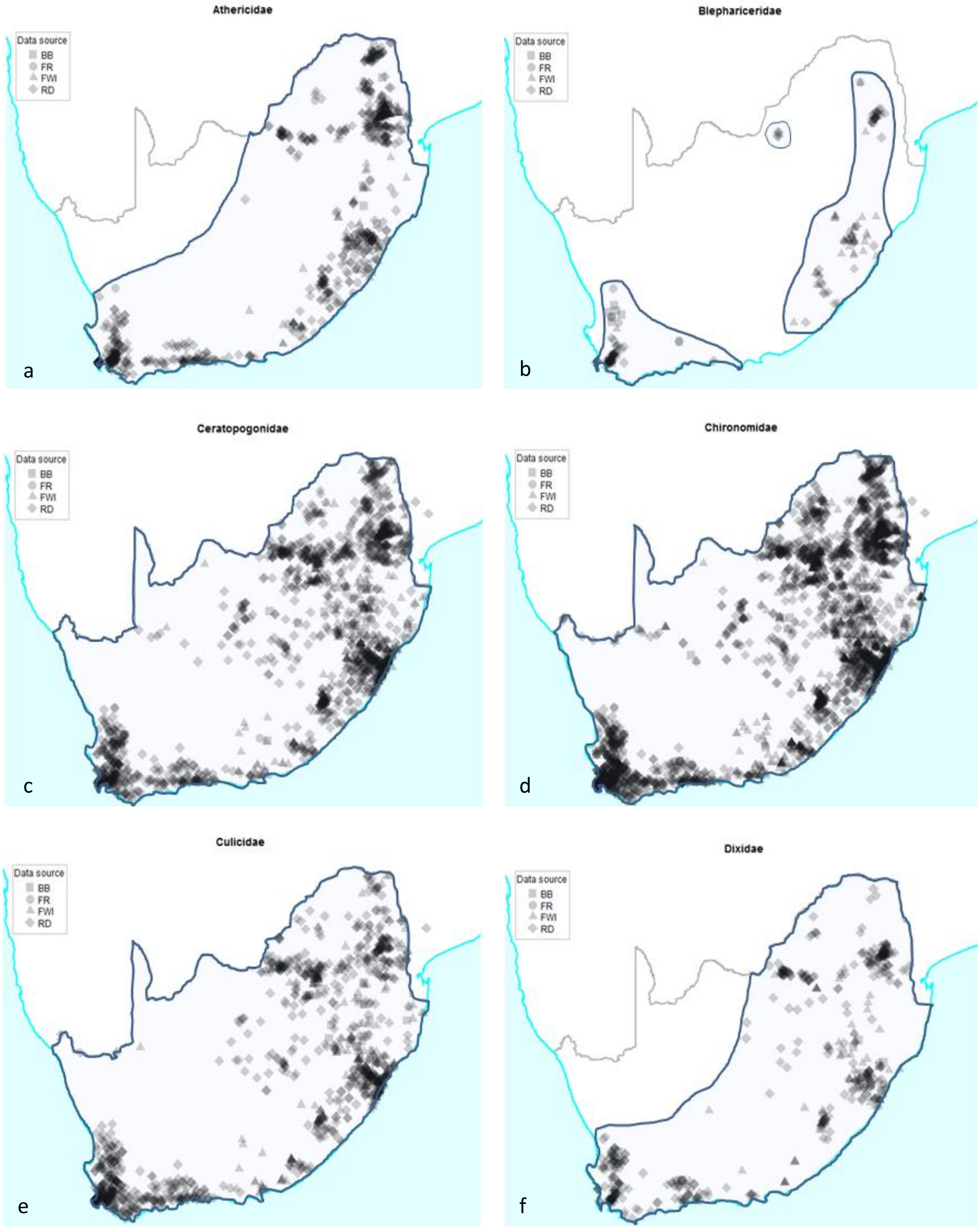

Figure 2.7: Distribution map of aquatic Diptera in South Africa: (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d) Chironomidae, (e) Culicidae, (f) Dixidae, (g) Empididae, (h) Ephydridae, (i) Muscidae, (j) Psychodidae, (k) Simuliidae, (l) Syrphidae, (m) Tabanidae, (n) Tipulidae.

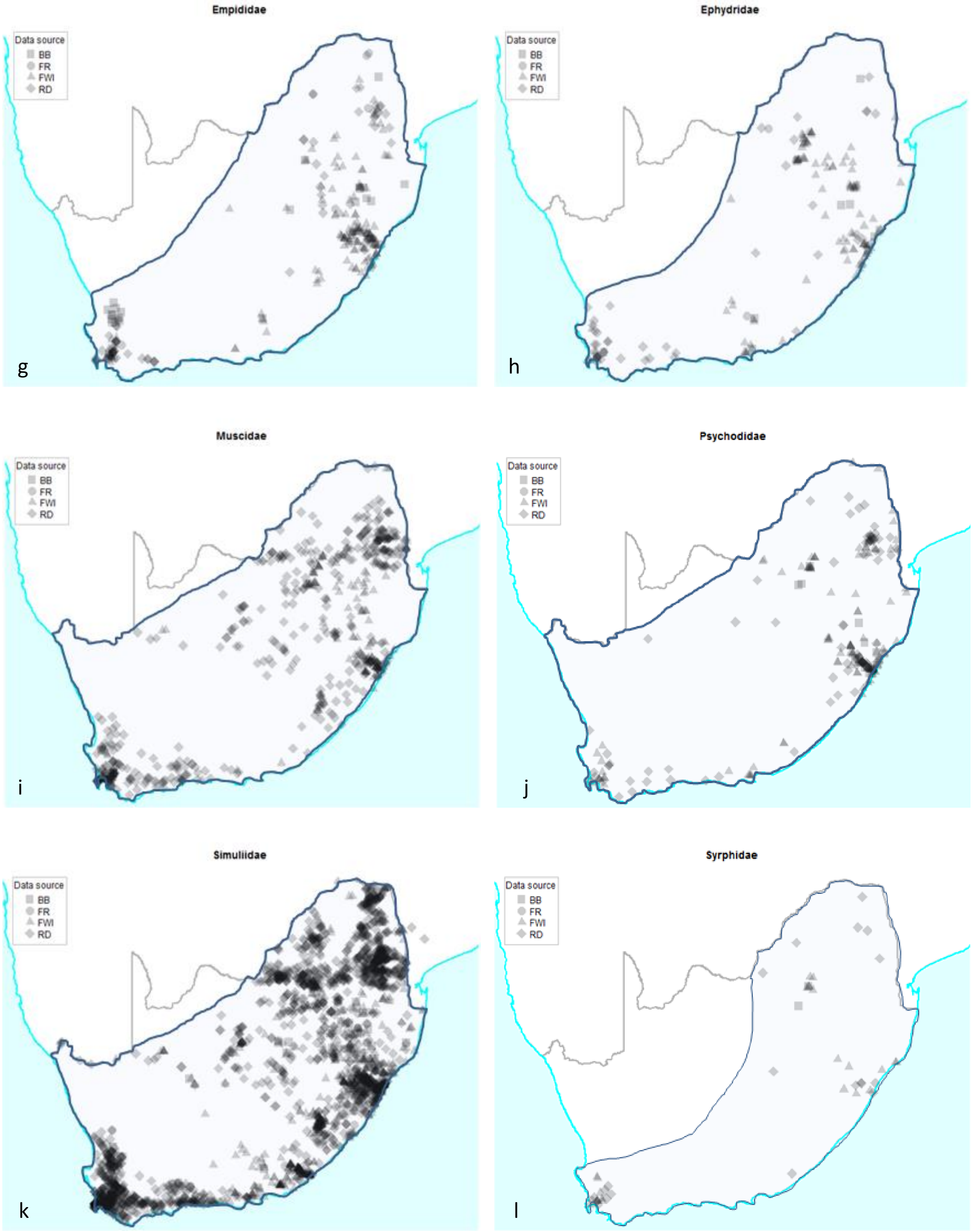

Figure 2.8 (continued): Distribution map of aquatic Diptera in South Africa: (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d) Chironomidae, (e) Culicidae, (f) Dixidae, (g) Empididae, (h) Ephydridae, (i) Muscidae, (j) Psychodidae, (k) Simuliidae, (l) Syrphidae, (m) Tabanidae, (n) Tipulidae.

Table 2.4: The distribution ranges of Diptera families based on distribution records obtained from the Biobase, Rivers Database, Freshwater Invertebrate Collection and a study to determine the environmental requirements of four different Insect orders.

	Diptera Family	Athericidae	Blephariceridae	Ceratopogonidae	Chironomidae	Culicidae	Dixidae	Empididae	Ephydridae	Muscidae	Psychodidae	Simuliidae	Syrphidae	Tabanidae	Tipulidae
	Altitude Range (m a.m.s.l.)	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-3500	0-2000	0-3000	0-3500
ZONE	Source zone			X	X						X	X			X
	High-gradient Mountain Stream	X	X	X	X	X	X	X	X	X	X	X		X	X
	Mountain Stream	X	X	X	X	X	X	X	X	X	X	X		X	X
	Transitional Zone	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Upper Foothills	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Lower Foothills	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Lowland	X		X	X	X	X	X	X	X	X	X	X	X	X
LEVEL I ECOREGION	Limpopo Plain	X		X	X	X	X			X	X	X		X	X
	Soutpansberg	X		X	X	X	X	X		X	X	X		X	X
	Lowveld	X		X	X	X	X	X	X	X	X	X	X	X	X
	North Eastern Highlands	X	X	X	X	X	X	X		X	X	X		X	X
	Northern Plateau			X	X	X					X	X			
	Waterberg	X		X	X	X	X	X		X		X	X	X	X
	Western Bankenveld	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Bushveld Basin			X	X	X			X	X		X		X	X
	Eastern Bankenveld	X		X	X	X	X	X		X	X	X		X	X
	Northern Escarpment Mountains	X	X	X	X	X	X	X	X	X	X	X		X	X
	Highveld	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Lebombo Uplands	X		X	X	X	X		X	X	X	X		X	X
	Natal Coastal Plain			X	X	X					X	X	X	X	X
	North Eastern Uplands	X		X	X	X	X	X	X	X	X	X	X	X	X
	Eastern Escarpment Mountains	X	X	X	X	X	X	X	X	X	X	X		X	X
	South Eastern Uplands	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	North Eastern Coastal Belt	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Drought Corridor	X		X	X	X		X	X	X		X		X	X
	Southern Folded Mountains	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	South Eastern Coastal Belt	X	X	X	X	X	X		X	X	X	X		X	X
	Great Karoo	X	X	X	X	X	X		X	X		X		X	X
	Southern Coastal Belt	X	X	X	X	X	X	X	X	X	X	X		X	X
	Western Folded Mountains	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	South Western Coastal Belt	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Western Coastal Belt	X		X	X	X	X			X		X		X	X
	Nama Karoo			X	X	X	X		X	X	X	X		X	X
	Namaqua Highlands	NO SAMPLES IN THIS ECOREGION													
	Orange River Gorge			X	X	X				X		X		X	X
	Southen Kalahari	X		X	X	X	X	X	X	X	X	X		X	X
	Ghaap Plateau			X	X	X				X		X		X	
	Eastern Coastal Belt	X	X	X	X	X			X	X	X	X	X	X	X

There are thirteen genera of Culicidae (mosquitoes) in southern Africa with no endemic genera to the region (Coetzee 2003). Mosquito larvae live in a wide range of habitats including temporary pools, artificial containers, dams, swamps and slow flowing streams and rivers (Coetzee 2003). The culicids have a widespread distribution throughout South Africa (Figure 5e), occurring in all the sampled ecoregions and all geomorphological zones (except the source zone) at altitudes up to 3500m a.m.s.l. (Table 2.4).

The Dixidae (meniscus midges) are a small family with a worldwide distribution and very little endemism at a generic level (Harrison *et al*. 2003c). All dixids have aquatic larvae and pupae with the larvae living near the water's edge at the surface (Barraclough and Londt 1985, Harrison *et al*. 2003c). According to Freeman (1956) there are eight species of Dixidae in two genera known from sub-Saharan Africa with three species known only from South Africa. This family occurs fairly widely throughout South Africa but even though they are absent from the dry areas of the Northern Cape and North West provinces (Figure 2.5 f) they have been recorded from the lower Orange River. This family has been recorded from all geomorphological zones except the source zone, at altitudes up to 3500 m. a.m.s.l. (Table 2.4).

The Empididae (dance flies) are a large family with a worldwide distribution. There are approximately 33 empidid genera known from southern Africa (Harrison *et al*. 2003a), but not all Empididae have aquatic forms. Eight empidid genera with aquatic species have been recorded from southern Africa (Smith 1980, Harrison *et al*. 2003b). The immature stages of the southern African Empididae are poorly known but have been found in a variety of habitats (Harrison *et al*. 2003b). Empidid pupae apparently do not produce cocoons and are found on submerged stones, stony crevices or sometimes in empty simuliid cocoons (Harrison *et al*. 2003b). The distribution range in Figure 2.5 g and Table 2.4 coincides mostly with that in Picker *et al*. (2003) with the family extending from the northern part of South Africa to the southern- and western Cape. This family has been recorded from all geomorphological zones except the source zone and at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The Ephydridae (shore flies and brine flies) are a large family that is well represented in most parts of the world (Harrison et al. 2003b). According to Cogan (1980) 32 ephydrid genera have been recorded from South Africa, although not all Ephydridae have aquatic larvae. Most ephydrids tend to live around freshwater environments such as ponds and streams although some occur near salt water (Barraclough and Londt 1985). Ephydridae are found in a wide variety of habitats with some species able to tolerate environmental extremes and these larvae can therefore be found in severely polluted water bodies (Cogan 1980). The distribution indicated in Figure 2.5 h and Table 2.4 coincides with that in Picker *et al.* (2003). Ephydridae have been recorded from all geomorphological zones except the source zone at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The Muscidae are regarded as the most abundant, widespread, commonly seen flies in South Africa (Barraclough and Londt 1985) but only the subfamily Limnophorinae are known to have fully aquatic larvae (Harrison et al. 2003b). The larvae of *Limnophora* are common in rivers in most regions with perennial rivers, whereas little is known of the larvae of *Lispe*, except that they usually occur at the margins of standing or slow-flowing water bodies (Harrison *et al*. 2003b). The distribution range in Figure 2.5 i coincides with that in Picker *et al*. (2003) with the family occurring throughout the whole of South Africa in all geomorphological zones except the source zone and at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The Psychodidae (moth flies, sewage flies) are a small family with an almost worldwide distribution (Harrison *et al*. 2003c), but only the Psychodinae subfamily have species with aquatic larvae. Sub-Saharan Africa has 16 genera of Psychodinae, with 23 species occurring in South Africa. Although Psychodidae are generally associated with organically polluted water the larvae of *Pericoma* are found in mountain waterfalls in the Cape Fold Belt and Drakensberg mountain ranges (Harrison *et al*. 2003c). The Psychodinae occur near water as their larvae live in water or moist soil (Barraclough and Londt 1985). According to Picker *et al.* (2003) this family occurs throughout the whole of South Africa. Although they can occur throughout the whole of South Africa, the localities tend to be clustered around areas that are polluted (Figure 2.5 j). The Psychodidae have been recorded from all geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The Simuliidae (blackflies or river midges) have an almost worldwide distribution that includes many species of economic, veterinary and medical importance. In spite of the almost cosmopolitan distribution, Simuliidae are excluded from certain oceanic islands and the Polar Regions (de Moor 2003). In the southern African region, there are about 65 blackfly species although the larvae of a number of species are undescribed (de Moor 2003). De Moor (2003) notes the presence of two genera in South Africa with the genus *Simulium* occurring throughout the whole of South Africa. The aquatic larvae of simuliids are filter feeders that are almost completely restricted to running waters (Barraclough and Londt 1985, de Moor 2003). The simuliid pupal cocoon can vary from an open ended “slipper” shape to a “shoe” shape with a closed protective ridge. The species with “slipper-shaped” cocoons are generally found in slower flowing water than those with “shoe-shaped” cocoons (de Moor 2003). According to Picker *et al.* (2003) Simuliidae occur throughout the whole of South Africa but from Figure 2.5 k and Table 2.4 it can be seen that they do not occur in the Kalahari Desert region near the border with Namibia and Botswana as there are only ephemeral and episodic rivers in this region which will not be suitable conditions for the simuliid larvae. Simuliidae have been recorded from all sampled ecoregions except the Northern Plateau and from all geomorphological zones at altitudes up to 3500 m a.m.s.l. (Table 2.4).

The larvae of the aquatic Syrphidae are more commonly known as rat-tailed maggots, because they have long telescoping posterior siphons enabling them to live in oxygen-poor polluted systems (Barraclough and Londt 1985, Harrison *et al*. 2003b). All seven genera known to have species with aquatic larvae occur in South Africa (Harrison *et al.* 2003b). According to Picker *et al.* (2003) this family occurs throughout the whole of South Africa but the current study looking only at the species with aquatic larvae indicates a more restricted distribution as is evident from Figure 2.5 l and Table 2.4 with no records in the dry central region of the Karoo, Northern Cape and western sections of the North West Province. The distribution of this family coincides to a large degree with the urban areas (Figure 2.5 l). The Syrphidae have not been recorded from the source zone, mountain streams and high gradient mountain streams but occur at altitudes up to 2000m a.m.s.l. (Table 2.4).

The Tabanidae (horse flies) are a cosmopolitan family of about 3000 species (Harrison *et al*. 2003b). Only about 250 of the Afrotropical tabanid species have been recorded from southern Africa (Chainey and Oldroyd 1980). Tabanid larvae live in wet places such as mud and damp soil usually at the margins of ponds, lakes and streams (Barraclough and Londt 1985). The few South African tabanid species with fully aquatic larvae belong mostly to the common world-wide genera (Harrison *et al*. 2003b). The distribution range of the Tabanidae includes the whole of South Africa (Picker *et al.* 2003), although the distribution range of the species with aquatic larvae as indicated in Figure 2.5 m and Table 2.4 excludes the Kalahari Desert region near the border with Namibia and Botswana. The Tabanidae have been recorded from all sampled ecoregions except the Northern Plateau and all geomorphological zones except the source zone and at altitudes up to 3000 m a.m.s.l. (Table 2.4).

The Tipulidae (craneflies) are the largest dipteran family with about 14 000 species worldwide. The larvae of many species are found in moist places with the larvae of only a few species found in freshwater habitats (Harrison *et al.* 2003c). Eight genera with aquatic larvae have been reported from southern Africa with the larvae usually occurring in well aerated fast flowing water (Harrison et al. 2003c). The Tipulidae have a similar distribution range to the Tabanidae in the sense that they occur throughout the whole of South Africa except for the Kalahari Desert region near the border with Namibia and Botswana (Figure 2.5n). Tipulidae have been recorded from all sampled ecoregions except the Northern Plateau, and from all geomorphological zones at altitudes up to 3500 m a.m.s.l (Table 2.4).

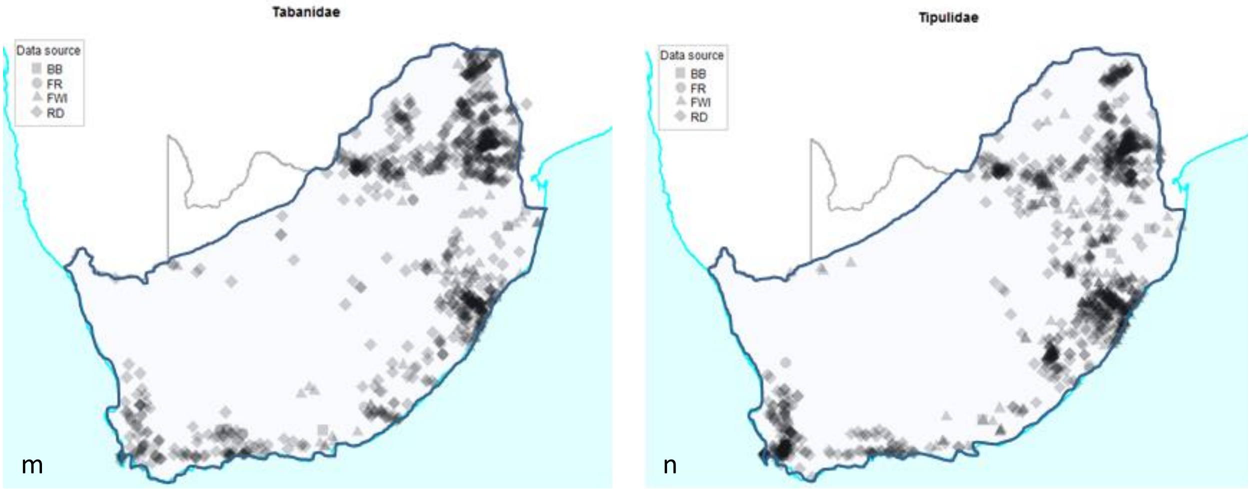

Figure 2.9 (continued): Distribution map of aquatic Diptera in South Africa: (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d) Chironomidae, (e) Culicidae, (f) Dixidae, (g) Empididae, (h) Ephydridae, (i) Muscidae, (j) Psychodidae, (k) Simuliidae, (l) Syrphidae, (m) Tabanidae, (n) Tipulidae.

2.4 Conclusions

Although by no means conclusive, this chapter gives a good indication of the distribution ranges of the families in the four orders under investigation. It also highlights the need to archive voucher specimens, not only for new or unidentified taxa, but also to validate the range distributions of known taxa. An example of this is the range extension in a number of taxa such as the Calamoceratidae (Trichoptera) and the Ptilodactylidae (Coleoptera) as well as the identification of questionable distribution records for a number of mostly south-western Cape endemics such as Barbarochthonidae, Sericostomatidae and Glossosomatidae. These questionable records can be investigated and corrected only if there are preserved specimens available. For the most part the questionable records are from the Rivers Database and no preserved specimens are available. It seems as if there are certain families such as the Oligoneuriidae, Teloganodidae, and particularly, a number of the cased caddisflies that are more prone to being misidentified than others. It is important that the SASS training courses offered in South Africa highlight these problems so that samplers are aware of the pitfalls. Another important aspect is to make samplers aware of the importance of lodging voucher specimens and to preserve unfamiliar taxa for proper identification by experts.

This chapter also highlighted the scarcity in distribution records of a number of families, most notably that of the Hydrosalpingidae (Trichoptera), Ptilodactylidae, Limnichidae (Coleoptera) and Ephemeridae (Ephemeroptera). There are a number of families with disjunct distributions such as: Prosopistomatidae (Ephemeroptera), Limnichidae (Coleoptera) and Blephariceridae (Diptera). Although the distribution of the Blephariceridae can be explained by their rather stringent habitat requirements the distribution of the Prosopistomatidae and the Limnichidae cannot be explained readily and might be more a function of insufficient collection rather than strict habitat requirements.

The hypothesis tested in this chapter was that the macroinvertebrate assemblage structure can be differentiated based on Ecoregion delineation and geomorphological zonation. The results indicated that this is true for certain taxa while other taxa have a countrywide distribution and have been recorded from most geomorphological zones. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on Ecoregion and geomorphological zone. The null hypothesis that the macroinvertebrate assemblage structure cannot be differentiated on Ecoregion delineation and geomorphological zonation is therefore rejected.

CHAPTER 3

ENVIRONMENTAL REQUIREMENTS OF SELECTED EPHEMEROPTERA, TRICHOPTERA, COLEOPTERA AND DIPTERA IN SOUTH AFRICA.

3.1 Introduction

Changes in the flow regime have a potential impact on aquatic ecosystem integrity. These changes are due, among others, to abstraction of water and release of water into a system, often as a result of economic development. In response to the ecological consequences of diminishing and altered flow regimes, a range of methodologies have evolved that attempt to quantify the Ecological Water Requirements (EWR) of rivers (Pollard 2002). Freshwater macroinvertebrates have been used to assess the biological integrity of stream ecosystems with relatively good success throughout the world (Resh 2008, Boon and Pringle 2009, Dallas *et al*. 2010, Buss *et al*. 2015, Kaaya *et al*. 2015), more commonly than any other biological group (O'Keeffe and Dickens 2000) because they offer a good reflection of the prevailing flow regime and water quality in a river. In addition they form an essential component of the riverine ecosystem (Allan 1995, Skoroszewski and de Moor 1999, O' Keeffe and Dickens 2000, Weber *et al.* 2004, Buss *et al*. 2015). Freshwater macroinvertebrates are important processors of transported organic matter in rivers and serve a vital function in purifying the water in a river and also provide a valuable food source for larger animals within and even outside the system (Allan 1995, Skoroszewski and de Moor 1999, O' Keeffe and Dickens 2000, Weber *et al.* 2004).

Distribution of a freshwater macroinvertebrate population is set by the tolerance of the individuals in the population to an array of environmental factors. The distribution pattern resulting from habitat selection by a given freshwater macroinvertebrate species reflects the optimal overlap between habit (mode of existence) and physical environmental conditions that comprise the habitat - substratum, flow and turbulence (Cummins 1993). Habitat functions as a temporally and spatially variable physical, chemical, and biological template within which freshwater macroinvertebrates can exist (Orth 1987, Poff and Ward 1990). Numerous studies have demonstrated the importance of physical habitat quantity and quality in determining the structure and composition of biotic communities (e.g. Modde *et al*. 1991, Aadland 1993, Ebrahimnezhad and Harper 1997). Habitat can also be defined as any combination of velocity, depth, substratum, physico-chemical characteristics and biological features that will provide the organism with its requirements for each specific life stage at a particular time and locality (Bovee 1982).

Macroinvertebrate distribution within the diversity of patches and habitats of rivers is determined by a number of factors typically acting on a localised scale (Covich *et al*. 1999, Malmqvist 2002). Populations of benthic animals reflect the hydraulic habitats of a river on a smaller scale than the riverbeds of pools and riffles (Yabe and Nakatsugawa 2004, Brooks *et al*. 2005). A study by Brooks *et a*l. (2005) demonstrated that small-scale differences in hydraulic conditions due to different combinations of velocity, depth and substratum roughness, played an important role in the spatial distribution of macroinvertebrate assemblages in riffles, and Mérigoux and Dolédec (2004) related macroinvertebrate assemblage distribution and composition to direct measurements of near-bed hydraulics. Suitable environmental conditions and resources (quantity, quality and timing) have to be available in order to sustain a viable long-term population (Colwell and Futuyma 1971, May and MacArthur 1972, Pianka 1974, Statzner and Higler 1986). Because a variety of factors, such as environmental conditions and resources, are required to meet the life history requirements of species, the success of aquatic organisms can be limited by a single factor or by a combination of factors (Hardy 2000). Since many aquatic organisms have specific habitat requirements, seasonal variation in these factors may lead to seasonal variation in the distribution and abundance of benthic macroinvertebrates. Variation in discharge often translates into differences in wetted perimeter, hydraulic conditions and biotope availability. For example, biotopes such as runs become riffles under low-flow conditions, and marginal vegetation may change from lotic to lentic (Bunn and Arthington 2002, Dallas 2004a). Temperature often varies with season and the life cycles of many aquatic organisms are cued to temperature. Temperature may also affect the rate of development, reproductive periods and emergence time of organisms. All organisms have a range of temperatures over which optimal growth, reproduction and general fitness occur, and temperatures outside this range may lead to the exclusion of taxa unable to tolerate extreme highs or lows (Hawkins *et al*. 1997).

Although aquatic ecologists intuitively know that different species occupy different habitat types in streams, there is a difference between this intuitive knowledge and the ability to quantify those habitat characteristics selected by an organism. It is the quantification of these characteristics that distinguishes Habitat Suitability Curves from natural history descriptions (Bovee 1986). Although macroinvertebrates are used to set environmental flows in South Africa and abroad, very limited information is available about their flow requirements (Brunke *et al.* 2001, Schael 2002, Jowett 2002a, Jowett 2002b, Clifford *et al*. 2004, Cassin *et al.* 2004, Hanquet *et al.* 2004, Kleynhans *et al.* 2005a). In southern Africa some information is available on certain Ephemeroptera in the Inkomati System (Matthew 1968) and some species occurring in the Lesotho Highlands (Skoroszewski and de Moor 1999). A more structured approach is required to determine macroinvertebrate flow requirements taking account of different life stages, ecoregions, seasonality and substratum. The concept of Habitat Suitability Curves (HSCs) was developed as part of the Instream Flow Incremental Methodology (IFIM) and the Physical Habitat Simulation System (PHABSIM) in the 1980s by researchers at the United States Fish and Wildlife Service (Bovee 1982, Bovee 1986). Since then it has become one of the most commonly used methods to describe the environmental requirements of aquatic organisms (Orth and Maughan 1983, Jowett *et al.* 1991, Conklin *et al.* 1996, Bovee *et al*. 1998, Vadas and Orth 2001, Vismara *et a*l. 2001, Jowett 2002a, Jowett 2002b, Strakosh *et a*l. 2003, Jowett *et al.* 2008, Li *et al.* 2009).

Habitat Suitability Curves are known by a variety of names (Habitat Suitability Criteria, Suitability Index Curves, Habitat Suitability Index Curves, Proportion of Use, Preference Curves and Selectivity Curves) with no standardised nomenclature (Jowett *et al.* 2008). Regardless of what it is called or how it is derived, suitability curves are intended to represent a functional relationship between an independent variable (depth, velocity, substratum, cover, etc.) and the response of a taxon to a gradient of the independent variable expressed on a scale from 0 to 1. The method for developing this relationship depends on a number of factors such as data availability, data analysis technique and professional (expert) judgement. According to Waddle (2012) the route taken to arrive at a particular HSC is irrelevant, to a certain degree, as long as it reflects the biological reality of the taxon and life stage adequately in terms of the study's objectives. Despite the debate in the literature (Vismara *et al*. 2001, Austin 2007, Mouton *et al*. 2009, Waddle 2012) with regard to the best curve development technique, the most important factor remains the basic principal that the final functional relationship must represent the known biology of the target organisms and life stage (Bovee 1986, Waddle 2012).

Suitability curves have been broadly categorized into the three different curve types:

a) Category 1 curves where literature and professional opinion is used, often from negotiated definitions using the Delphi technique (Waddle 2012). These curves can be developed reasonably quickly and cheaply (Li *et al*. 2009).
b) Category 2 curves or utilisation functions are based on the frequency distributions of habitat attributes measured at locations used by the target organisms (Waddle 2012). These curves do not necessarily describe an organism's physiological or behavioural preferences because the preferred conditions may be rare within the stream where the data were collected (Waddle 2012).
c) Category 3 curves or preference functions are designed to reduce the bias associated with habitat availability by factoring out the influence of limited habitat choice. This aims to increase the transferability of the curves to different types of streams and conditions to those where the curves were originally developed (Waddle 2012).

This chapter focused on the ecological requirements of four insect orders used during the determination of the ecological Reserve. Members of the Ephemeroptera (e.g. Heptageniidae, Tricorythidae), Trichoptera (e.g. Hydropsychidae, Philopotamidae), Coleoptera (e.g. Elmidae, Psephenidae) and Diptera (e.g. Simuliidae, Chironomidae) are often used as indicators during Ecological Water Requirement- (Skoroszewski and de Moor 1999, Skoroszewski 2006, Brown *et al*. 2009) or Instream Flow Requirement (Harby *et al*. 2004, Biggs *et al*. 2008) studies throughout the world. It was therefore decided to concentrate on these four orders. Although Plecoptera are also often used, South Africa has only two plecopteran families and it was decided to rather concentrate on the more diverse orders that would also include families that are representative of the whole suite of environmental conditions in South Africa's rivers. The main aim of this chapter is to determine the preferred ranges of water depth, velocity as well as the substratum types required by these macroinvertebrate taxa.

3.2 Materials and Methods

3.2.1 Study area

A total of 266 samples were collected from 52 sites (Figure 3.1) between July 2005 and February 2009. These sites are spread throughout the country covering 19 Level I and 38 Level II Ecoregions (Kleynhans *et al*. 2005b). The altitudes range from 11 to 1793 m a.m.s.l and are located in a variety of geomorphological zones (Rowntree *et al.* 2000, Moolman 2008) ranging from Mountain Streams through to Lowland Rivers.

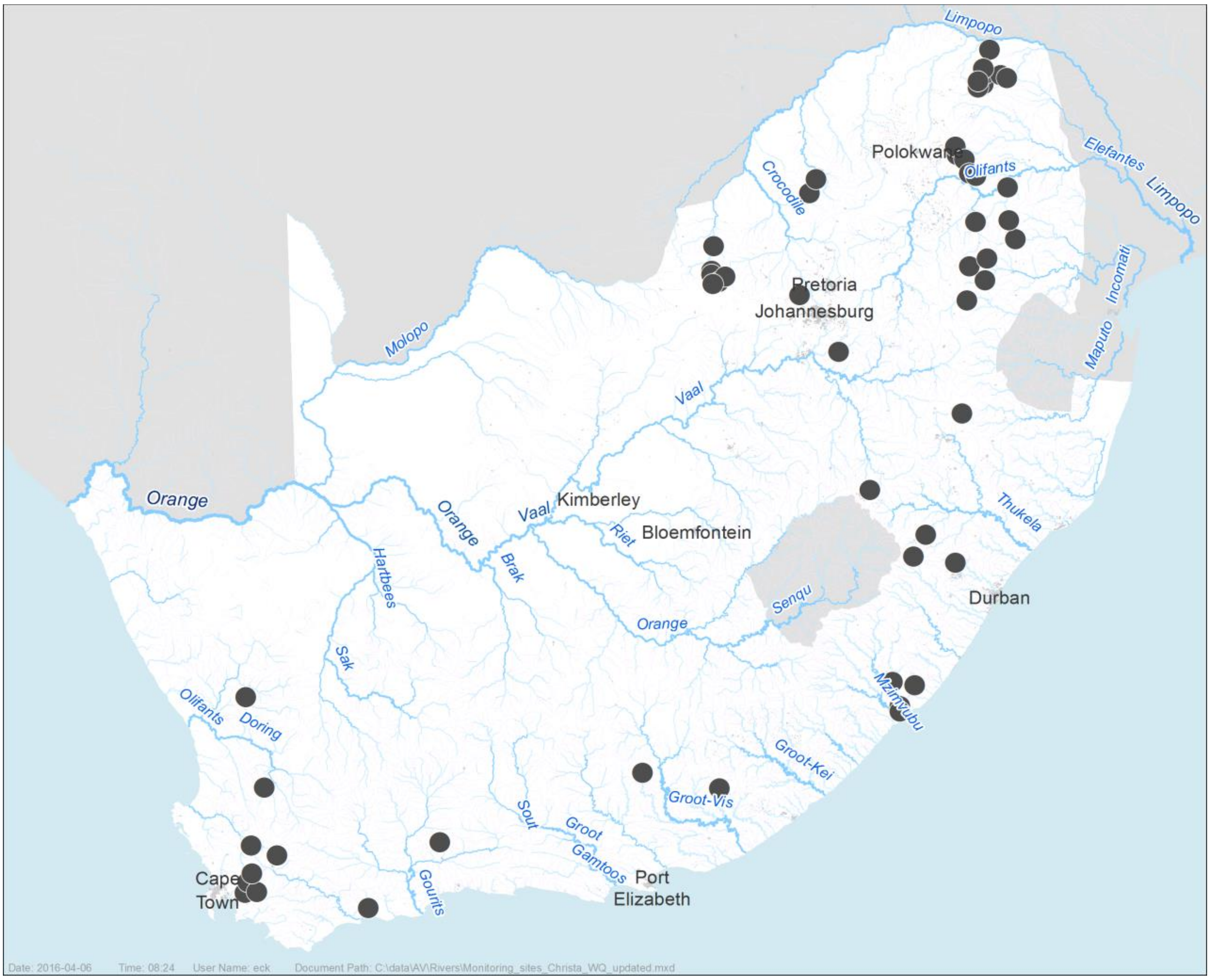

Figure 3.1: Map of the study area indicating sampling sites, major rivers and towns

The macroinvertebrates were collected using a Surber sampler (30 cm x 30 cm with a 0.5 mm mesh net) but the marginal vegetation was sampled with a hand net (30 cm x 30 cm with a 1 mm mesh). Samples were taken at a number of localities at each site to cover all substratum types, velocity and depth groupings. The water depth was measured at each locality and the velocity measured using an OTT C2 small current meter with 50 mm diameter propeller and Z30 counter, at 5 to 10 cm intervals (depending on the depth) as close to the bottom as practical up to the surface. The stones (20 to 750 mm diameter) were measured after brushing the macroinvertebrates off them. Basic *in situ* water measurements (temperature, dissolved oxygen, pH and Electric Conductivity) were collected at each site using an YSI model 556 MPS handheld multiparameter water quality meter. The macroinvertebrates were preserved in 80% ethanol and identified to family level using a Zeiss Stemi SV 11 Stereo Microscope and suitable identification keys (Barber-James and Lugo-Ortiz 2003, de Moor and Scott 2003, Day *et al*. 2003, Stals and de Moor 2008). Voucher specimens of the macroinvertebrate taxa will be lodged at the Albany Museum once all data analysis has been completed.

3.2.2 Data Analysis

Redundancy analysis (RDA) was done using Canoco 5.04 (ter Braak and Šmilauer 2012) to determine the environmental factors contributing most to the distribution of the different invertebrate families. A constrained RDA was first done on 261 samples, 43 taxa and 17 environmental variables. A total of 76 samples were excluded due to missing values in the explanatory data. The results from this RDA were then used to develop a subset of environmental variables, which was used to do another constrained RDA with the eight environmental variables that explained most of the variation.

The substrata were converted into categories, based on notes taken during the sampling and the medium “stone” diameter, using a modification of the system described in Bovee (1986) to facilitate further analyses (Table 3.1). Results from the RDA were then used to draw response curves for the selected families using the Species Response Curve function in Canoco 5.04 (ter Braak and Šmilauer 2012). A Generalised Additive Model (GAM) with Poisson distribution and log link function (family as the response and depth, velocity at 60% of depth and substratum category as predictors) were used with stepwise selection using the Akaike Information Criterion

(AIC) and two degrees of freedom (2 DF) factor to smooth the curves. Significance of relationships was regarded as $p < 0.05$.

The HSCs for the selected taxa were determined using the methods described in Bovee (1986) and Jowett et al. (2008). Separate univariate curves were developed for frequency and abundance and the average of the two curves was then used to derive the final HSC. A second order polynomial regression was done for the depth and velocity curves using Excel 2010. No regression was done for the substratum curves as they represent discrete categories rather than a range. Based on the reasoning of Jowett *et al*. (2008), only families with at least 20 individuals and that were present in at least 10 samples were selected for the HSCs. The families excluded from further analyses are indicated in bold in Tables 3.2 -3.5.

Table 3.1: Substratum scale used, modified from Bovee (1986)

Substratum category	Type	Particle size (mm)
1	Vegetation	
2	Mud	0.00024-0.062
3	Sand	0.062-2
4	Gravel	2-32
5	Pebble	32-64
6	Small Cobble	64-128
7	Large Cobble	128-256
8	Boulder	256-512
9	Bedrock	>512

3.3 Results

3.3.1 Redundancy Analysis (RDA)

Environmental (explanatory) factors in the RDA (Figure 3.2) explain a total 32.3% of the observed variation with the first axis explaining 14.27% and the second axis 20.15% of the variation. The RDA results indicated that velocity, pH, temperature, latitude, and longitude as well as, Ecoregion, geomorphological zone and substratum were determining factors for the distribution of the invertebrate families under consideration (Figure 3.2). The cobble substratum explained most (4.8%, $p < 0.05$) of the variation, followed by Ecoregion: other (4.5%, $p < 0.05$) and average velocity (4.1%, $p < 0.05$). Temperature (3.8%, $p < 0.05$), longitude (3.5%, $p < 0.05$), Ecoregion 9.03 (3.5%, $p < 0.05$), latitude (3.4%, $p < 0.05$) and Ecoregion 10.01 (3.2, % $p < 0.05$) also contributed to the explanation. The other factors with a significant ($p < 0.05$) contribution to explaining the distribution of the invertebrate families are: pH (2.6%), Ecoregion 24.06 (2.3%), Ecoregion 31.01 (1.9%). Ecoregions 21.01 and 17.01 as well as geomorphological zone D (upper Foothills) and sand each contributed 1.6% to the explanation.

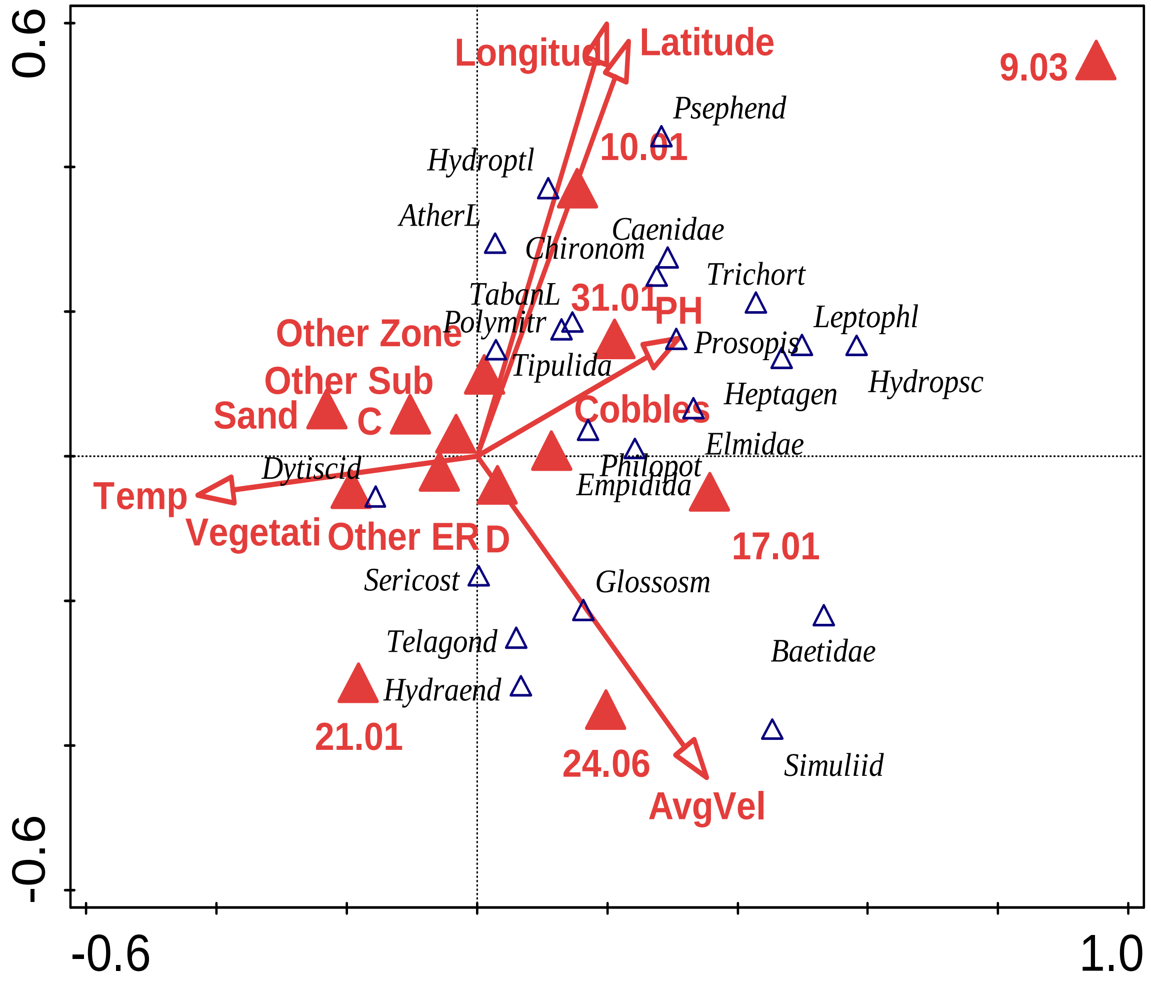

Figure 3.2: Redundancy Analysis performed on the abundance data of the invertebrate families and environmental variables. Macroinvertebrate taxa are indicated by black open triangles, while the explanatory variables are indicated in red. The triplot represents 32.3% of the variation in the data set, with 14.27 being explained on the first axis and 20.5% on the second axis. (Avg Vel = average velocity; Sub = Substratum; ER = Ecoregion)

The distribution of the Simuliidae (blackflies) was most closely explained by high velocity and that of Dytiscidae by the presence of vegetation and higher temperatures. A number of the families (Hydropsychidae, Elmidae, Heptageniidae, Leptophlebiidae, Philopotamidae, Empididae, Prosopistomatidae and Tricorythidae) were closely associated with the presence of cobbles. Psephenidae showed a close affinity to the Eastern Bankenveld (9.03) and Northern Escarpment Mountains (10.01) Ecoregions, while the distribution of the southern- and western Cape endemic Teloganodidae, Sericostomatidae and Glossosomatidae were associated with the South Western Coastal belt and the Great Karoo Ecoregions.

3.3.2 Generalised Additive Models (GAM)

Generalised Additive Models (GAMs) were developed for six ephemeropteran (Figure 3.3), five trichopteran (Figure 3.4), seven coleopteran (Figure 3.5) and eight dipteran families (Figure 3.6).

The GAM results for the Ephemeroptera were significant ($p < 0.05$). Baetidae, Heptageniidae Teloganodidae and Tricorythidae displayed similar depth response curves with the preferred depths around 30 cm, whereas Caenidae seem to prefer either shallow (<15 cm) or deep (>45cm) water and Leptophlebiidae exhibit a preference for shallow (<15 cm) water (Figure 3.3 a). Baetidae occur mostly at velocities less than 1.5 m/sec but do not seem to have a strong preference for any specific velocity although the largest response occurs at 0.6 m/s, whereas Caenidae seem to prefer the slower velocities (<0.3 m/s) (Figure 3.3 b). Heptageniidae and Leptophlebiidae show the greatest response at 0.6 m/s while Teloganodidae and Tricorythidae seem to prefer faster water with the greatest response at 1 m/s (Figure 3.3 b). Baetidae occur on all available substrata, although there seem to be a slight preference for larger substrata (Figure 3.3 c). Caenidae show the greatest response to the sand with the occurrences linked to the gravel, sand and mud (GSM) grouping (Figure 3.3 c). The other three mayfly families: Heptageniidae, Teloganodidae and Tricorythidae all show greatest response to the Cobble Pebble grouping although the Leptophlebiidae and Teloganodidae also occur in the sand and gravel biotopes (Figure 3.3 c).

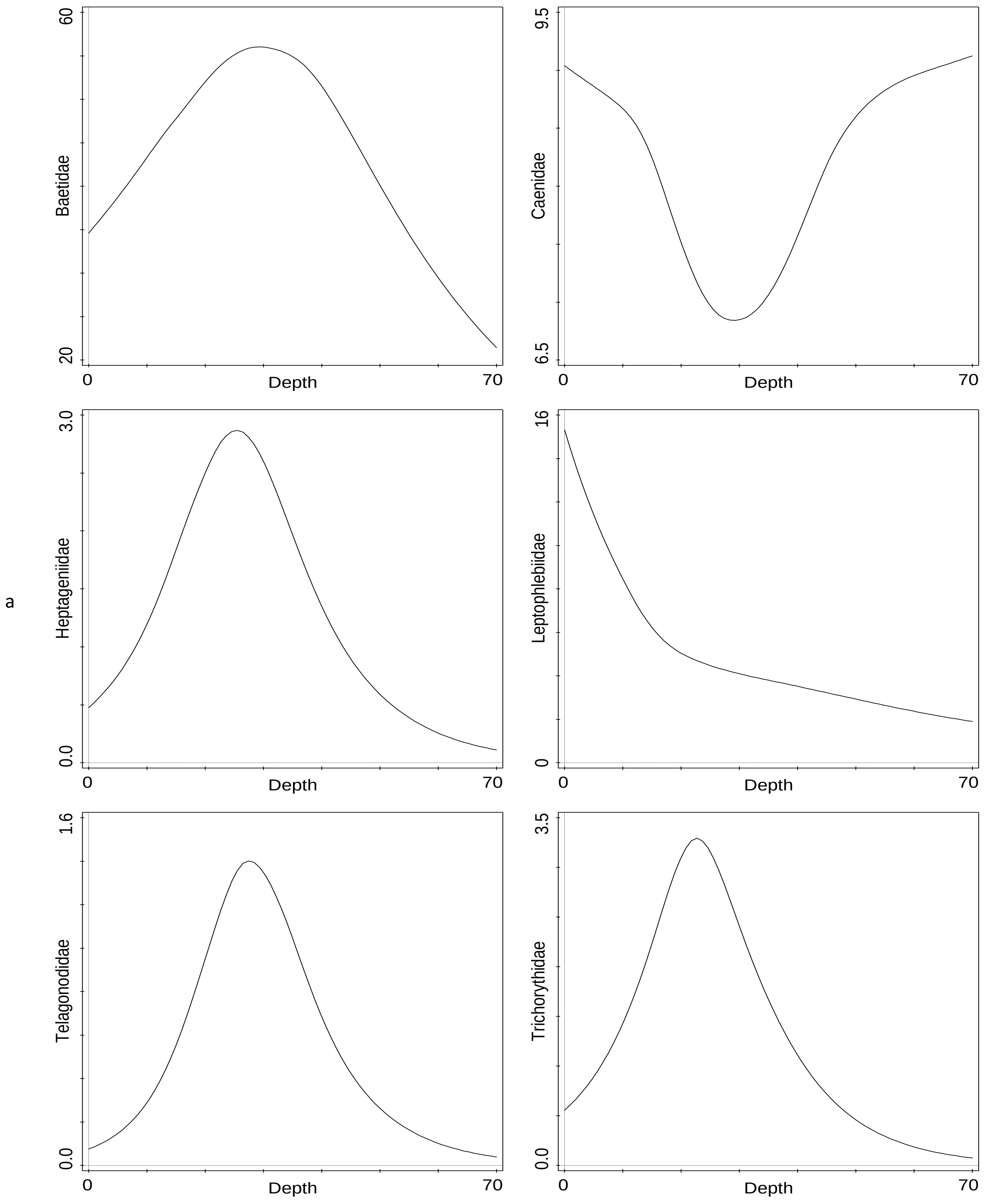

Figure 3.3: Generalised Additive Model graphs indicating the response of Ephemeroptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

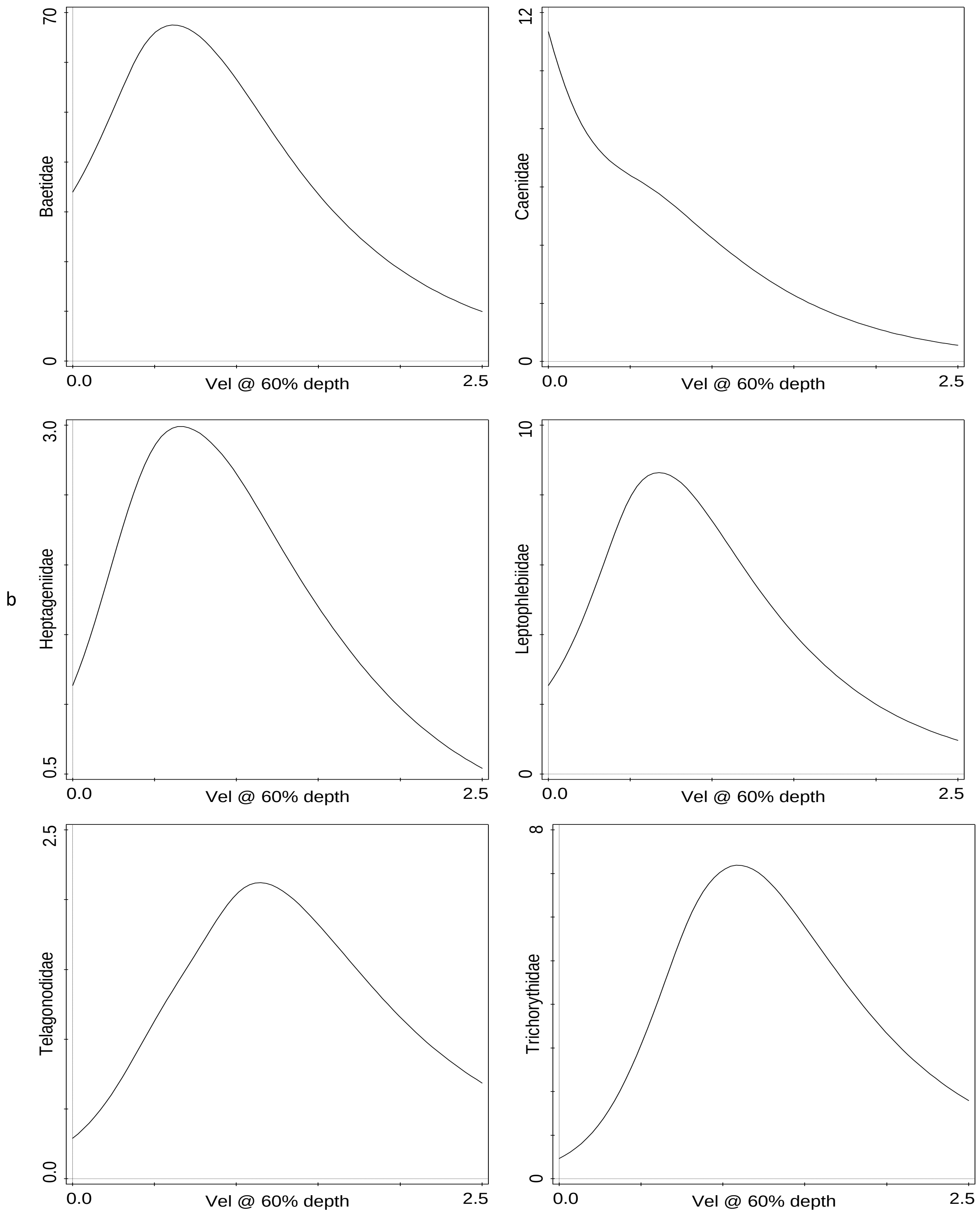

Figure 3.4 (continued): Generalised Additive Model graphs indicating the response of Ephemeroptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

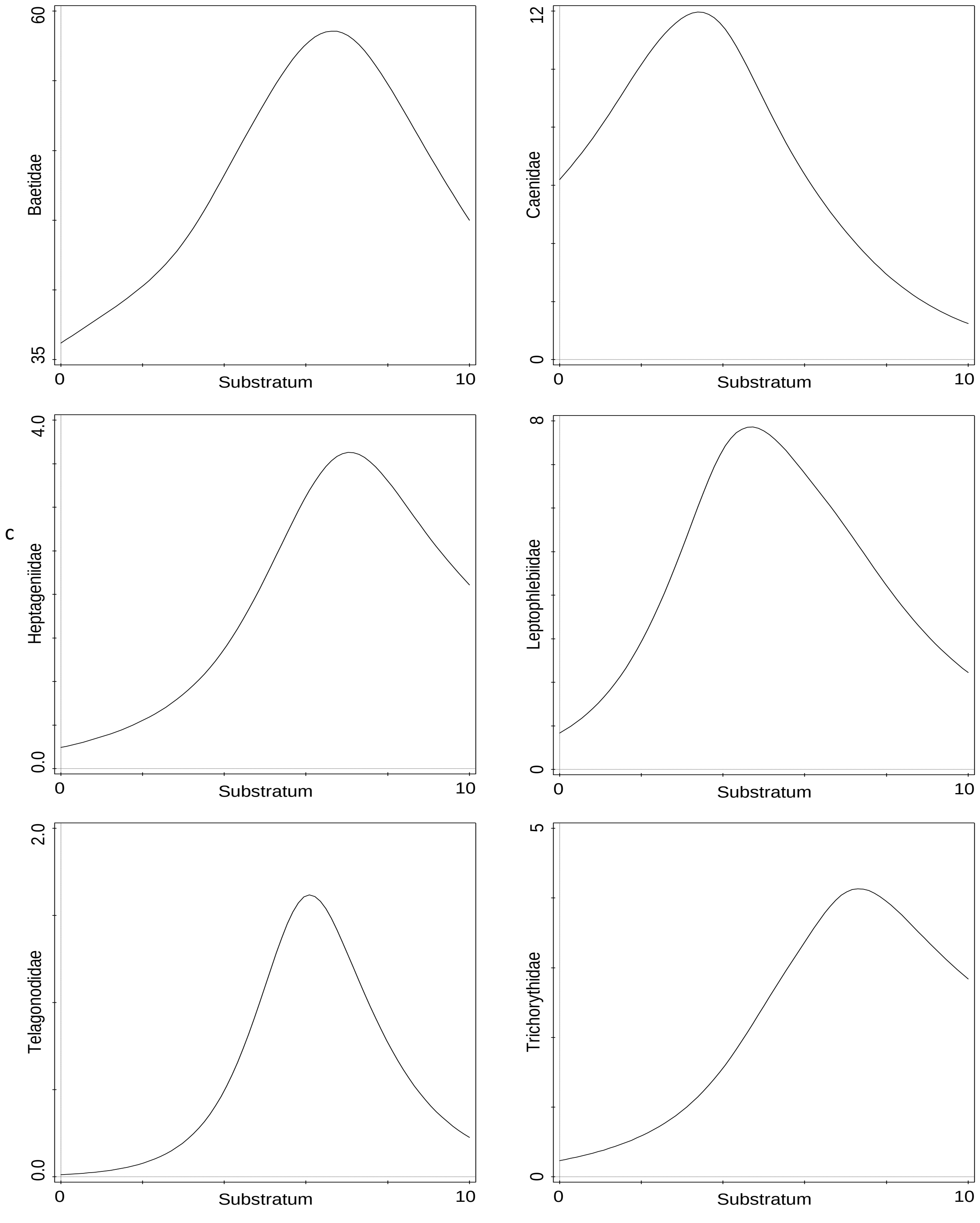

Figure 3.5 (continued): Generalised Additive Model graphs indicating the response of Ephemeroptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum

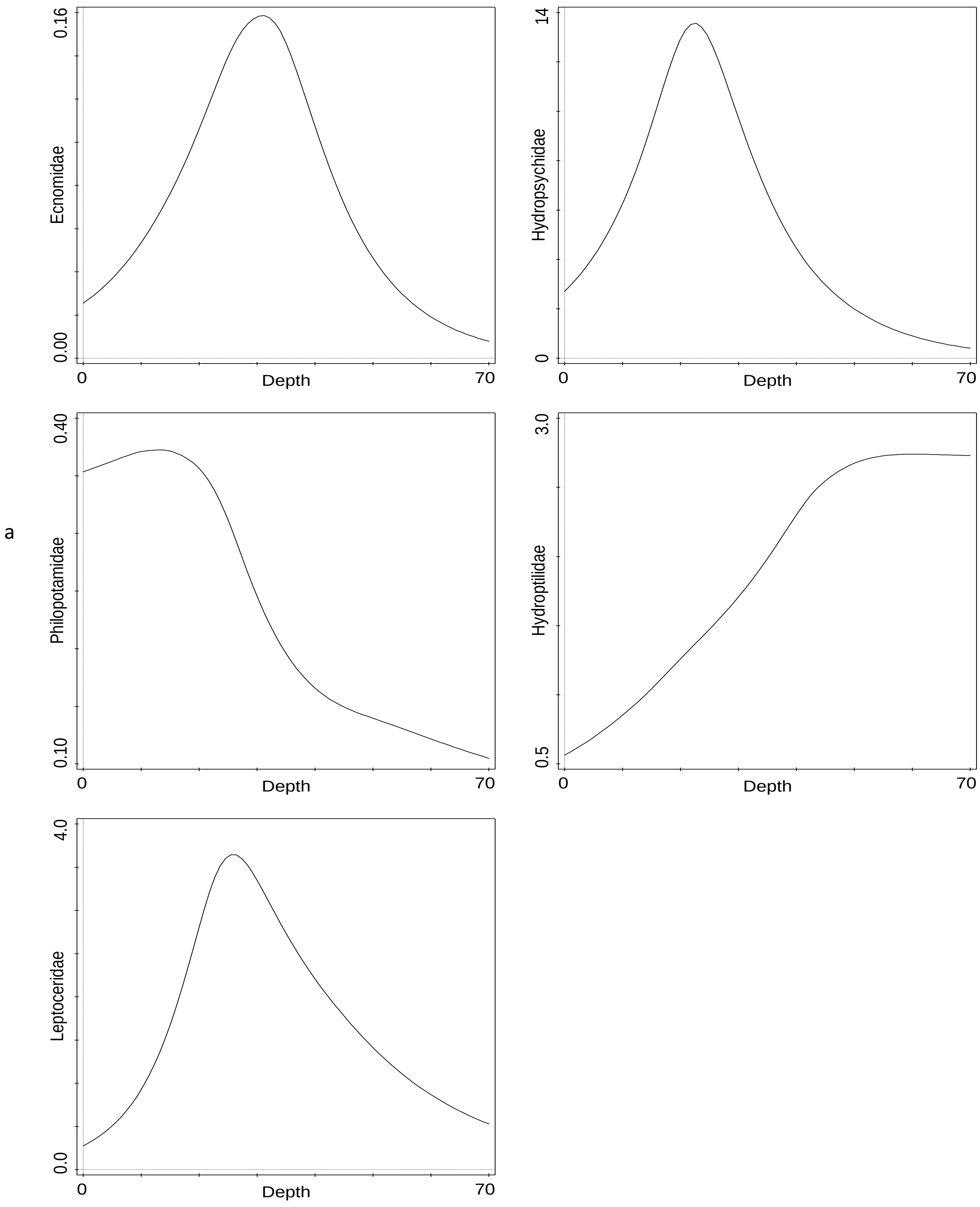

Figure 3.6: Generalised Additive Model graphs indicating the response of Trichoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

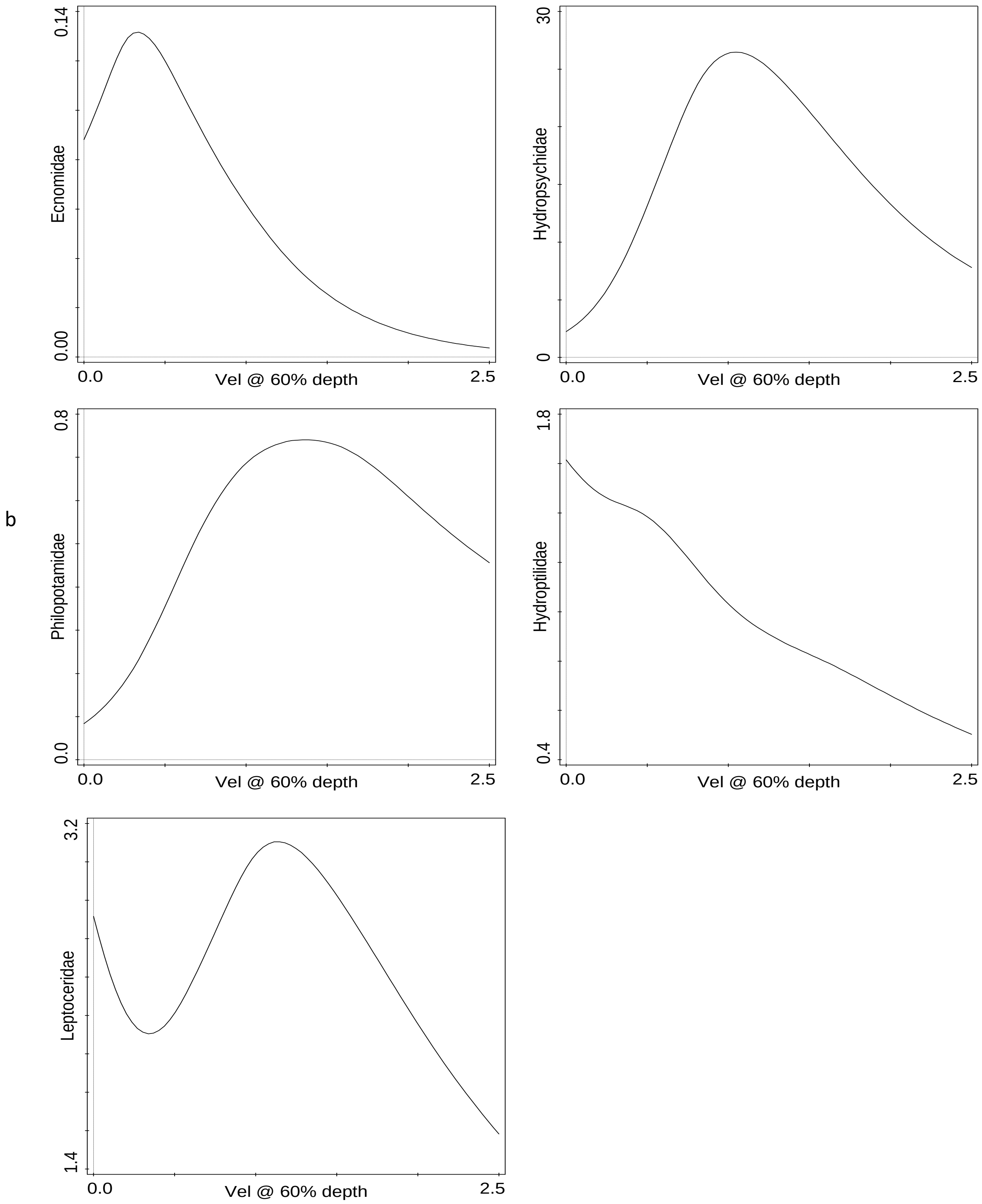

Figure 3.7 (continued): Generalised Additive Model graphs indicating the response of Trichoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

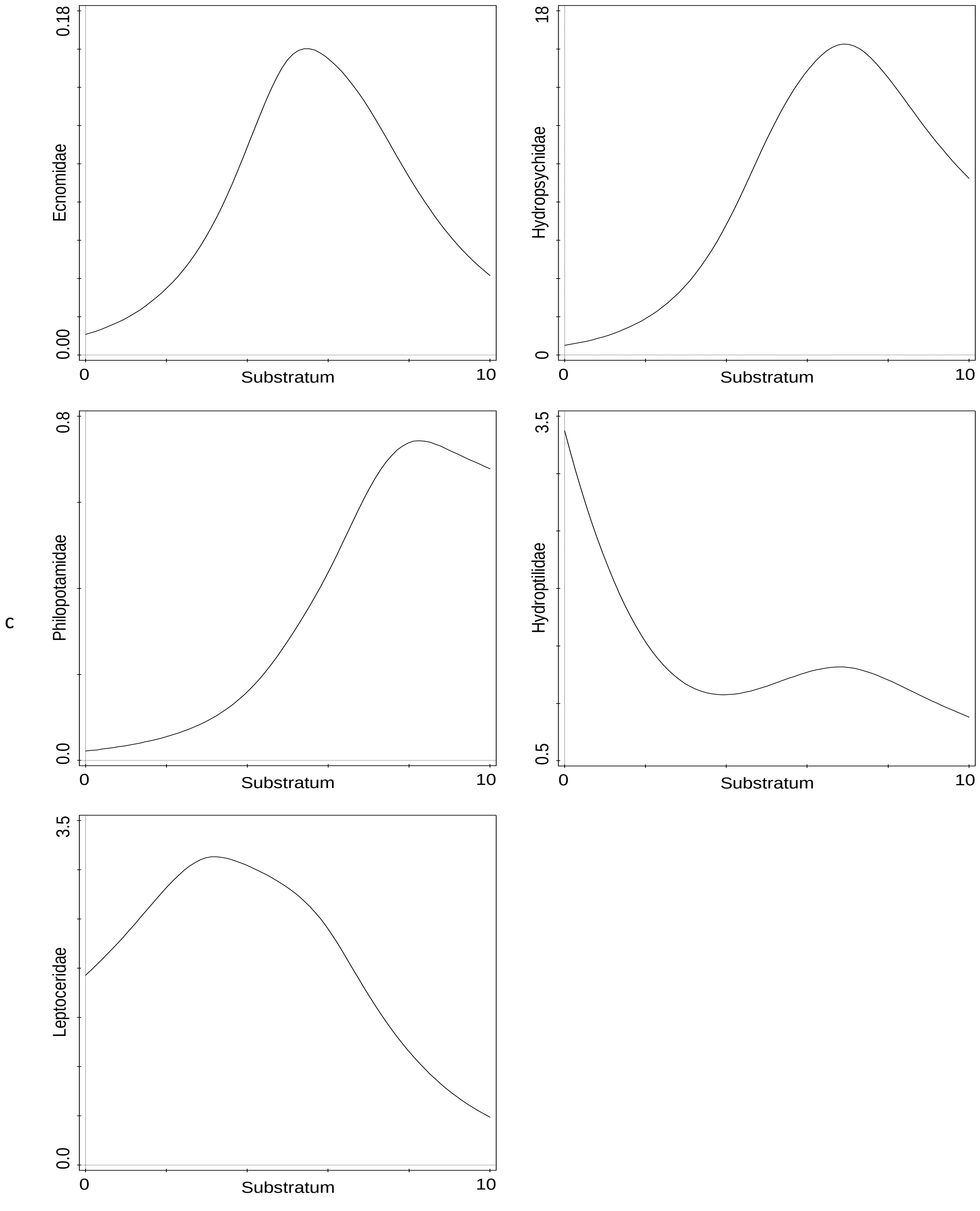

Figure 3.8 (continued): Generalised Additive Model graphs indicating the response of Trichoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

The GAM results for the Trichoptera did not display the same significant relationships as found for the Ephemeroptera, i.e. for the Ecnomidae (depth: $p < 0.05$, velocity: $p > 0.5$, substratum $p < 0.05$), Philopotamidae (depth: $p < 0.05$) and Hydroptilidae (velocity: $p < 0.05$). Ecnomidae ($p < 0.05$), Hydropsychidae and Leptoceridae had the greatest response to depths between 15 - 40 cm, while Philopotamidae ($p < 0.05$) preferred depths less than 30 cm and Leptoceridae depths greater than 30cm (Figure 3.4 a). The Ecnomidae's response to velocity is not significant ($p > 0.05$) but the greatest effect is at 0.4 m/s (Figure 3.4 b). Hydropsychidae and Philopotamidae indicate a preference for fast (>0.6 m/s) flowing water, while Hydroptilidae prefer slow (<0.3 m/s) flowing water and Leptoceridae exhibiting a bimodal response (figure 3.4 b). Ecnomidae ($p < 0.05$), Hydropsychidae and Philopotamidae all seem to prefer the Cobble biotope, while Hydroptilidae have a preference for vegetation and Leptoceridae were found in all biotopes although they seem to have a greater preference for the GSM (Figure 3.4 c). The wider preference range of Leptoceridae in particular is likely a function of the case types and hence species sampled.

Dytiscidae and Hydraenidae seem to prefer depths between 20 and 40 cm, Elmidae are found mostly at shallower depths (<20 cm) while Gyrinidae ($p<0.05$), Hydrophilidae and also Scirtidae ($p<0.05$) are more prevalent in deeper water (>50 cm) (Figure 3.5). Psephenidae ($p<0.05$) occur at a wide range of depths (>15 cm) with no clear preference for either deep or shallower water (Figure 3.5 a). Dytiscidae are the only Coleopteran family with a clear preference for standing (<0.1 m/s) water, while Elmidae, and Hydraenidae occur mostly in very fast (>0.6 m/s) flowing water (Figure 3.5 b). Hydrophilidae ($p > 0.05$), and Psephenidae are prevalent along a wider range of velocities (0.3-1.5 m/s) (Figure 3.5 b). Although the response is not significant ($p > 0.05$), Scirtidae seemed to be associated with deeper water (Figure 3.5 a). Velocity was not a suitable indicator for Gyrinidae (Table 3.4). Dytiscidae were found mostly in the GSM samples, but also seems to have an affinity with Vegetation (Figure 3.5 c). Hydraenidae, Psephenidae and Elmidae prefer the cobble biotope although Elmidae have a greater affinity for the pebble and small cobble substrate (Figure 3.5 c). Gyrinidae, Scirtidae and Hydrophilidae were mostly found in the Vegetation biotope (Figure 3.5 c).

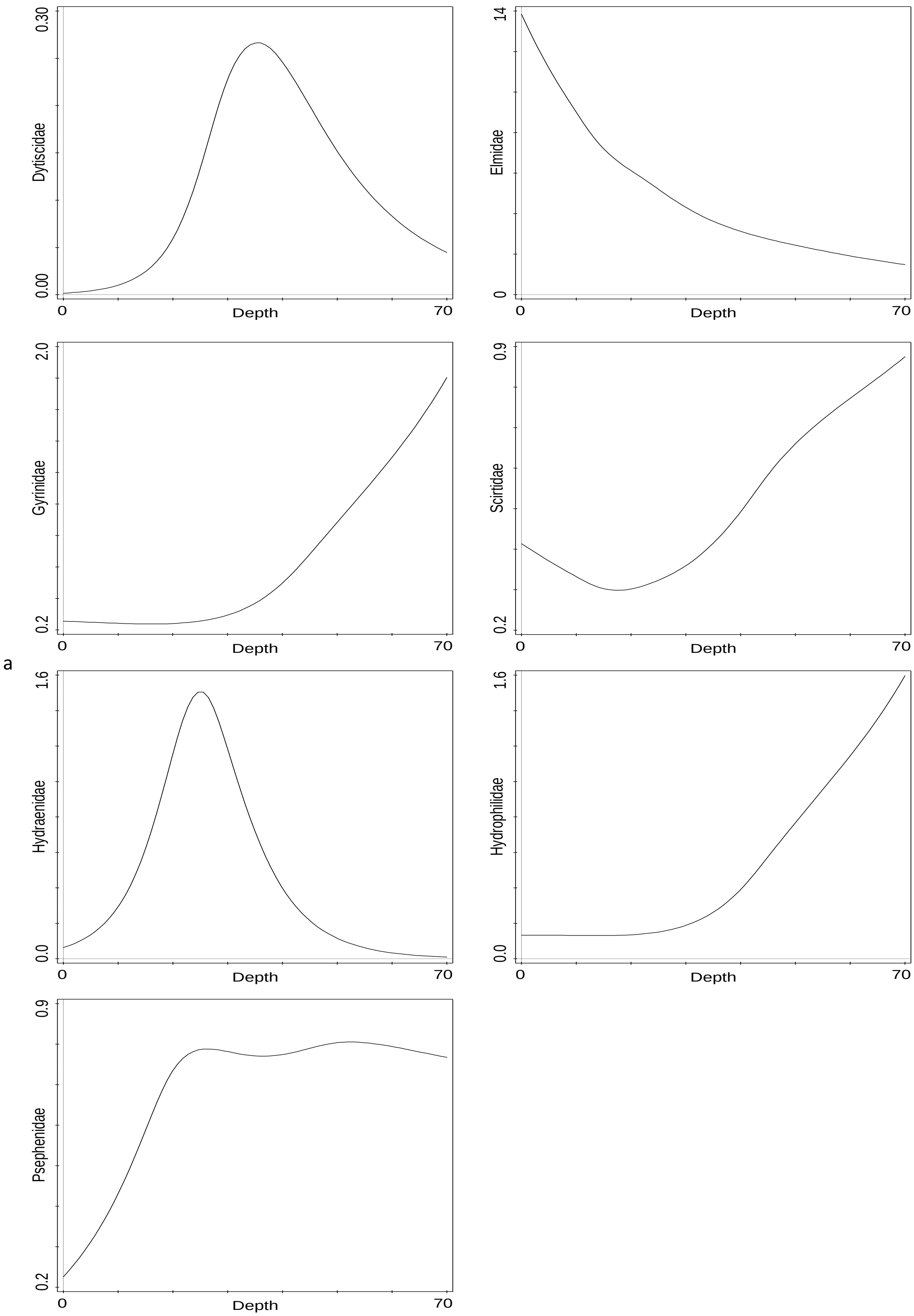

Figure 3.9: Generalised Additive Model graphs indicating the response of Coleoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

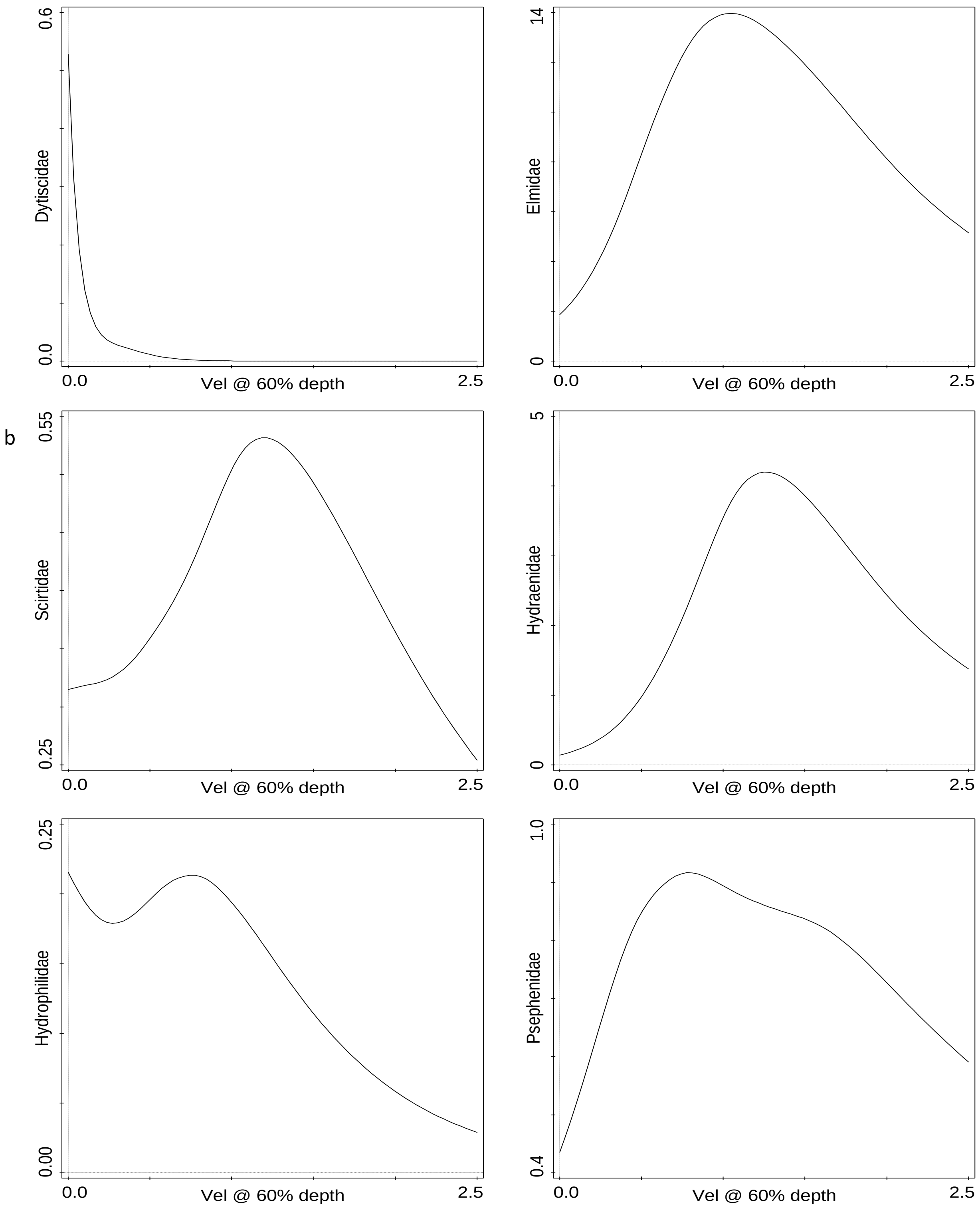

Figure 3.5 (continued): Generalised Additive Model graphs indicating the response of Coleoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

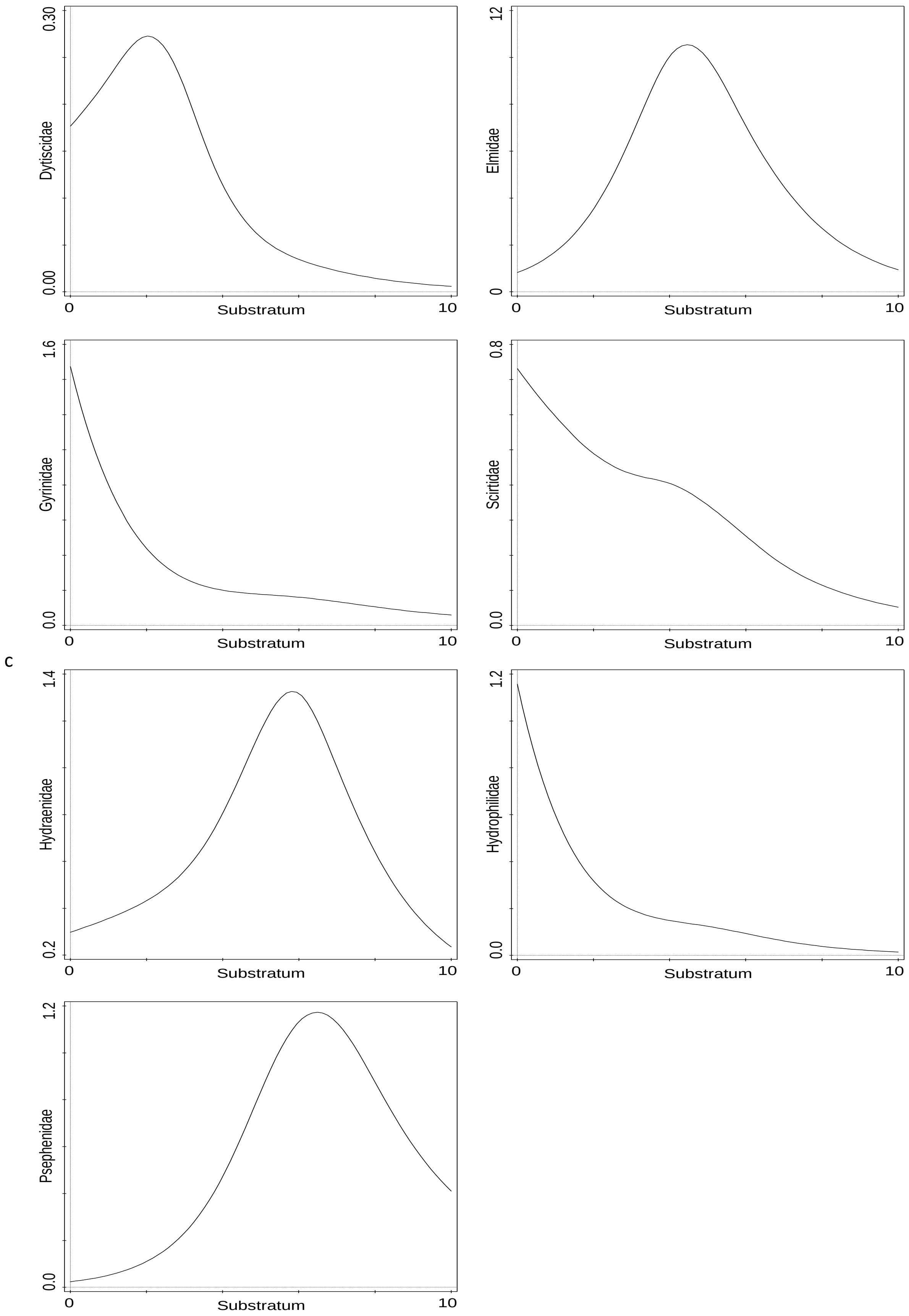

Figure 3.5 (continued): Generalised Additive Model graphs indicating the response of Coleoptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

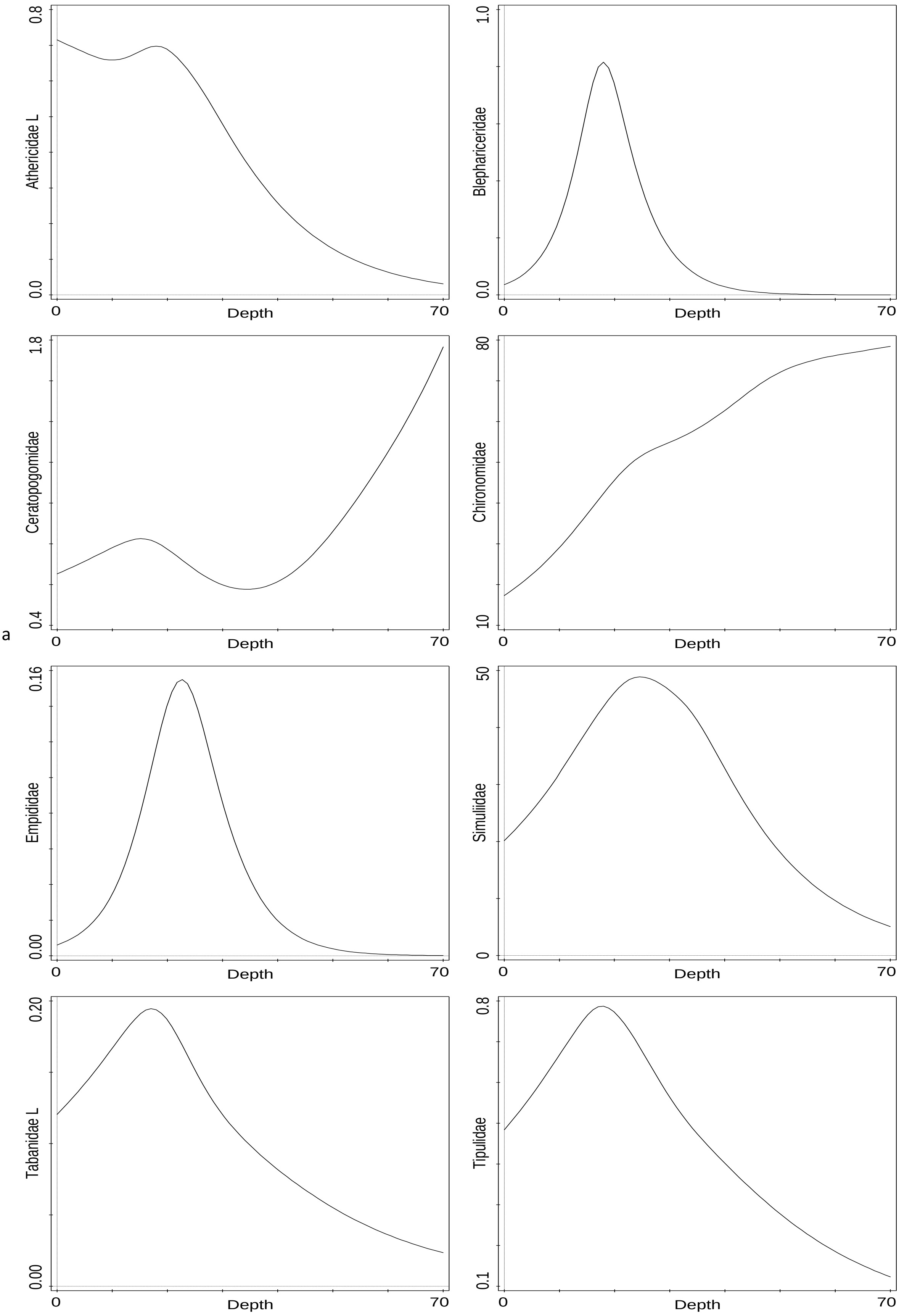

Figure 3.10: Generalised Additive Model graphs indicating the response of Diptera to: (a) Depth, (b) Velocity at 60% of the depth depth (vel@ 60%) and (c) Substratum.

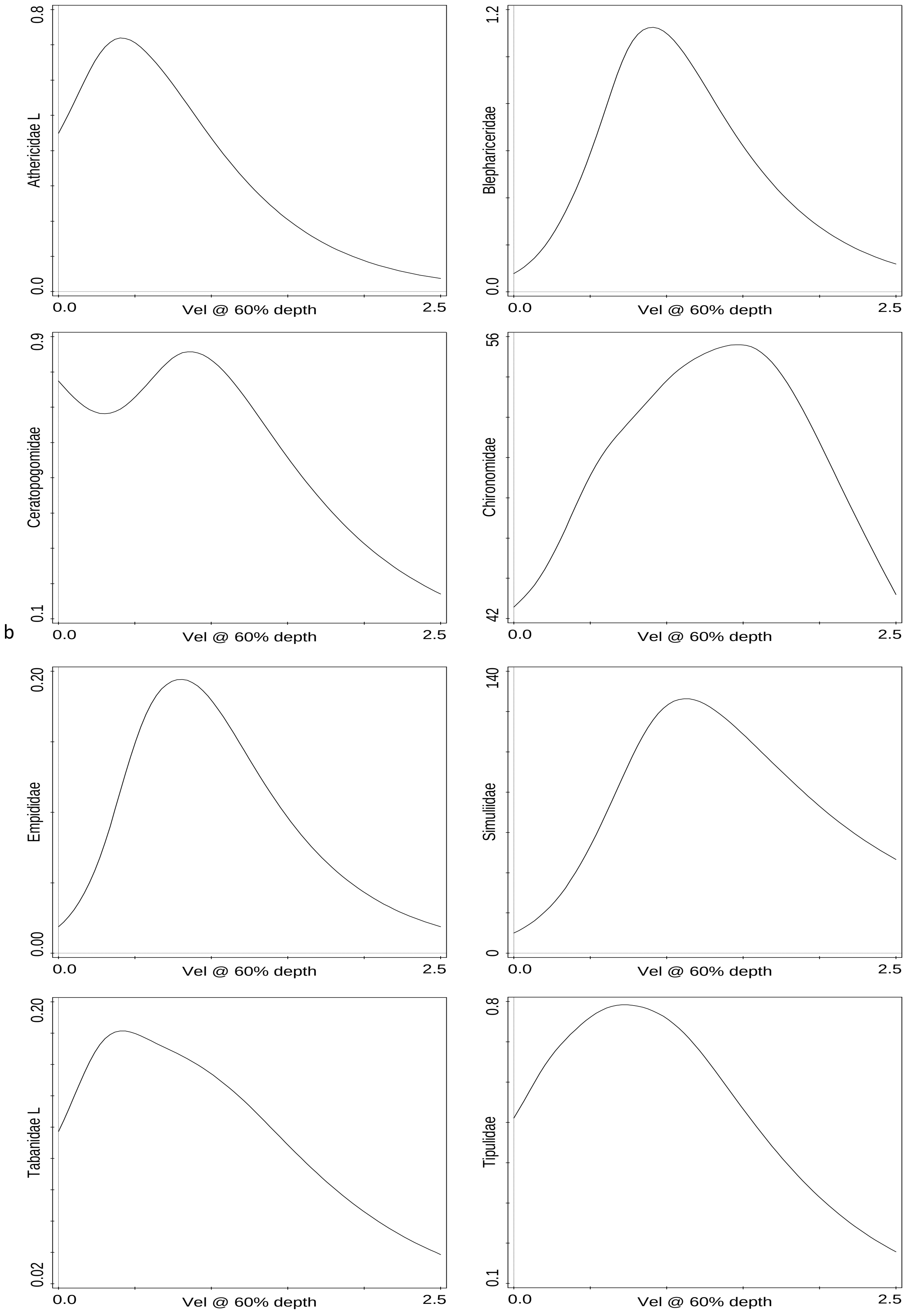

Figure 3.6 (continued): Generalised Additive Model graphs indicating the response of Diptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

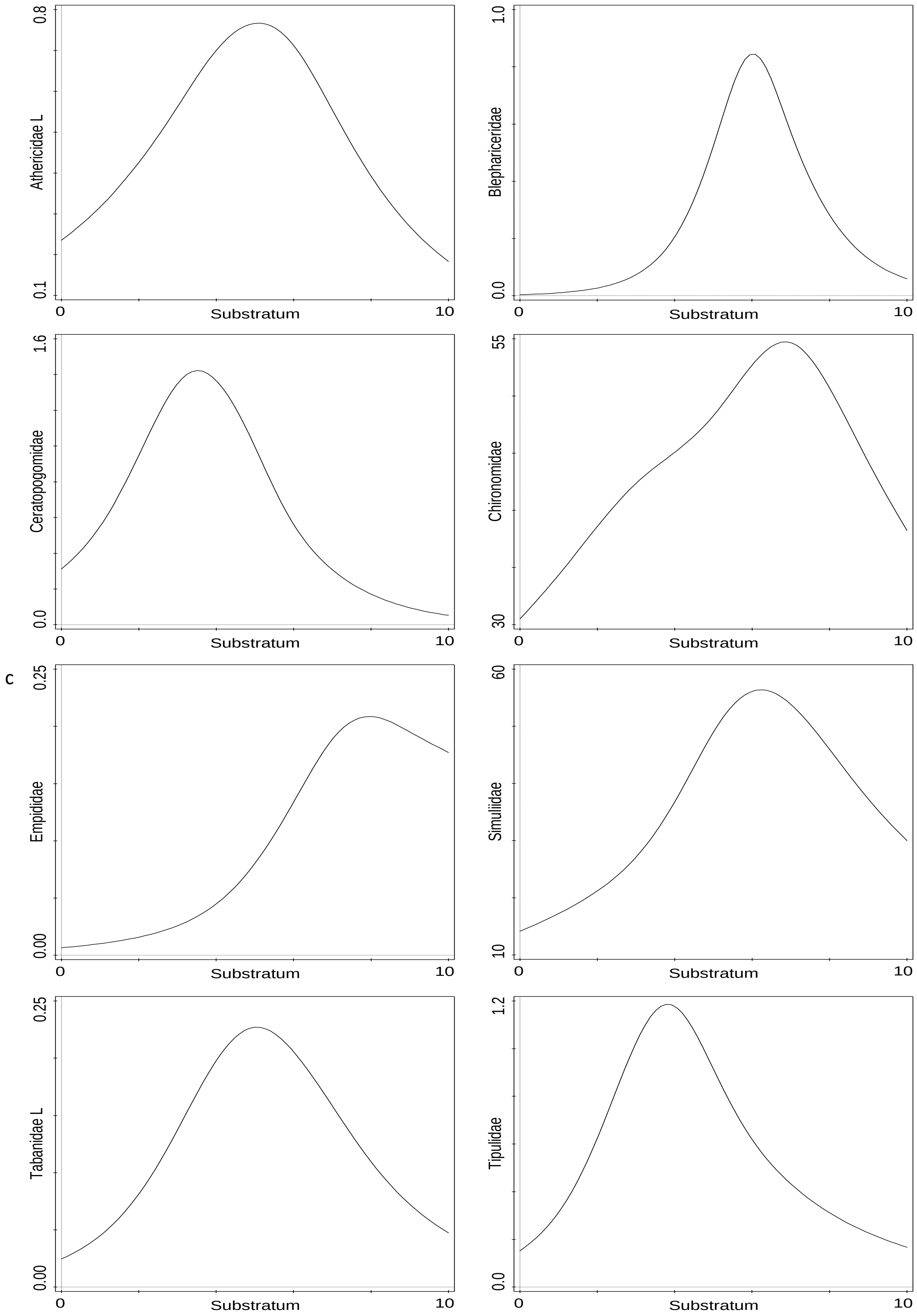

Figure 3.6 (continued): Generalised Additive Model graphs indicating the response of Diptera to: (a) Depth, (b) Velocity at 60% of the depth (vel@ 60%) and (c) Substratum.

Athericidae occurred mostly in the shallow (<30 cm) water, while the Chironomidae and Ceratopogonidae were mostly found in deeper (>30 cm) water (Figure 3.6 a). Blephariceridae and Empididae occurred in 10 - 30 cm deep water (Figure 3.6 a). Simuliidae occurred at a wide range of depths but mostly in 10 - 40 cm deep water (Figure 3.6 a). The Tabanidae ($p > 0.05$) and Tipulidae ($p < 0.005$) both preferred shallow (5 - 30 cm) water (Figure 3.6 a). Ceratopogonidae ($p < 0.05$), Chironomidae, Tabanidae ($p > 0.05$) and Tipulidae ($p < 0.05$) did not exhibit a clear preference for velocity (Figure 3.6 b). Athericidae and Empididae occurred mostly in slow to fast (0.1 - 1 m/s) water, while Blephariceridae and Simuliidae occurred in very fast (>0.6 m/s) flowing water (figure 3.6b). Athericidae occurred on substrata ranging from sand to large cobbles with the greatest response to the gravel substratum (Figure 3.6 c). Ceratopogonidae and Tipulidae occurred mostly in the GSM biotope while Chironomidae showed no clear preference for grain size and occurred in all substratum categories (Figure 3.6 c). Blephariceridae, Simuliidae and Tabanidae ($p < 0.05$) preferred Cobbles (Figure 3.6 c).

3.3.3 Habitat Suitability Curves (HSCs)

Summarised information about the numbers of samples, sites and specimens collected for each of the families are indicated in Tables 3.2 - 3.5. Habitat Suitability Curves (HSCs) were developed for six ephemeropteran- (Figure 3.7), five trichopteran- (Figure 3.8), seven coleopteran- (Figure 3.9) and eight dipteran families (Figure 3.10). These HSCs presented in Figures 3.7 – 3.10 include separate curves based on frequency, average abundance as well as a combined curve consisting of the average values for the frequency and abundance curves. Where enough data were available separate curves were developed for the larval and adult Coleoptera as well as the larval and pupal Diptera. A second order polynomial regression was then performed on the combined curve and the goodness of fit (R2) and significance (F) indicated in Tables 3.2 – 3.5 and Figures 3.7 – 3.10.

Table 3.2: Summary of the number of samples, sites and specimens for different Ephemeroptera families. No Habitat Suitability Curves were determined for the families indicated in bold typeface.

Family	No of Samples	No of sites	No of Specimens	R^2 Depth	Significance (f) Depth	R^2 Velocity	Significance (f) Velocity
Batidae	249	53	13159	0.9396	0.0604	0.6794	0.2354
Caenidae	139	45	1999	0.1465	0.8535	0.9952	0.0536
Heptageniidae	65	29	536	0.8681	0.1319	0.9636	0.0419
Leptophlebiidae	133	24	1389	0.9493	0.0507	0.7532	0.3740
Machadorythidae	**1**	**1**	**1**	N/A	N/A	N/A	N/A
Oligoneuridae	**2**	**1**	**2**	N/A	N/A	N/A	N/A
Polymitarcyidae	**2**	**2**	20	N/A	N/A	N/A	N/A
Prosopistomatidae	**1**	**1**	**1**	N/A	N/A	N/A	N/A
Teloganodidae	20	6	220	0.9588	0.0417	0.8384	0.5085
Trichorythidae	49	27	598	0.83548	0.1652	0.9799	0.0823

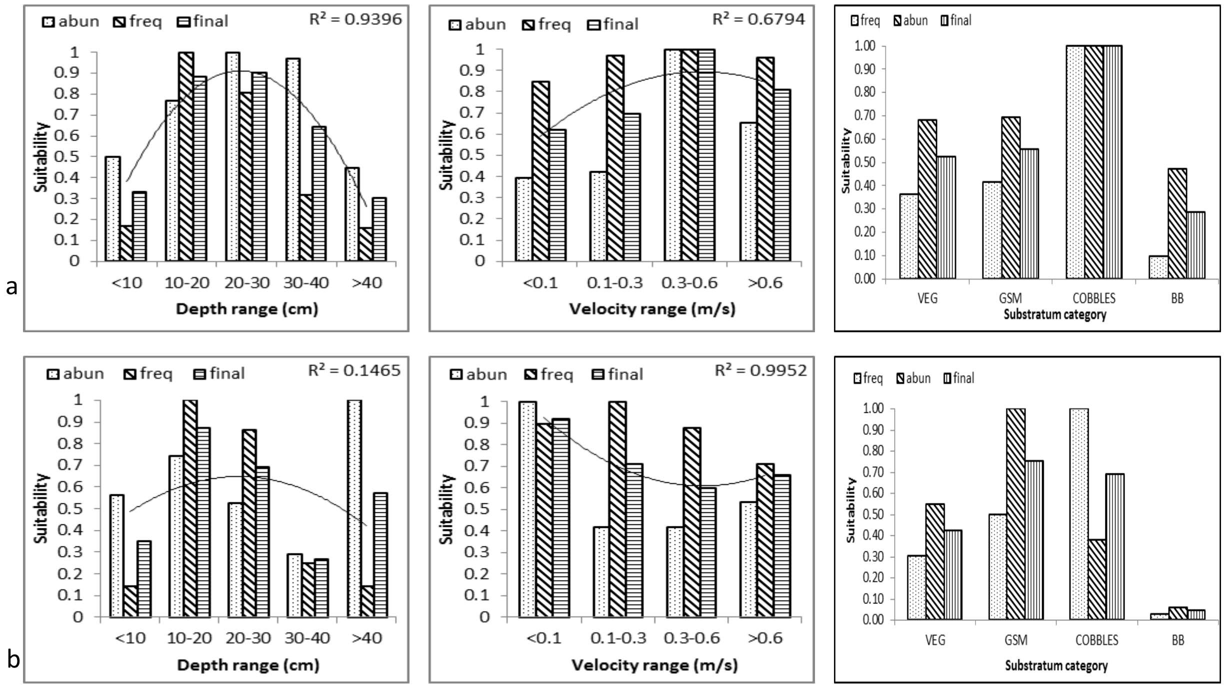

Figure 3.11: Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Ephemeroptera (a) Baetidae, (b) Caenidae, (c) Heptageniidae, (d) Leptophlebiidae, (e) Teloganodidae, and (f) Tricorythidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

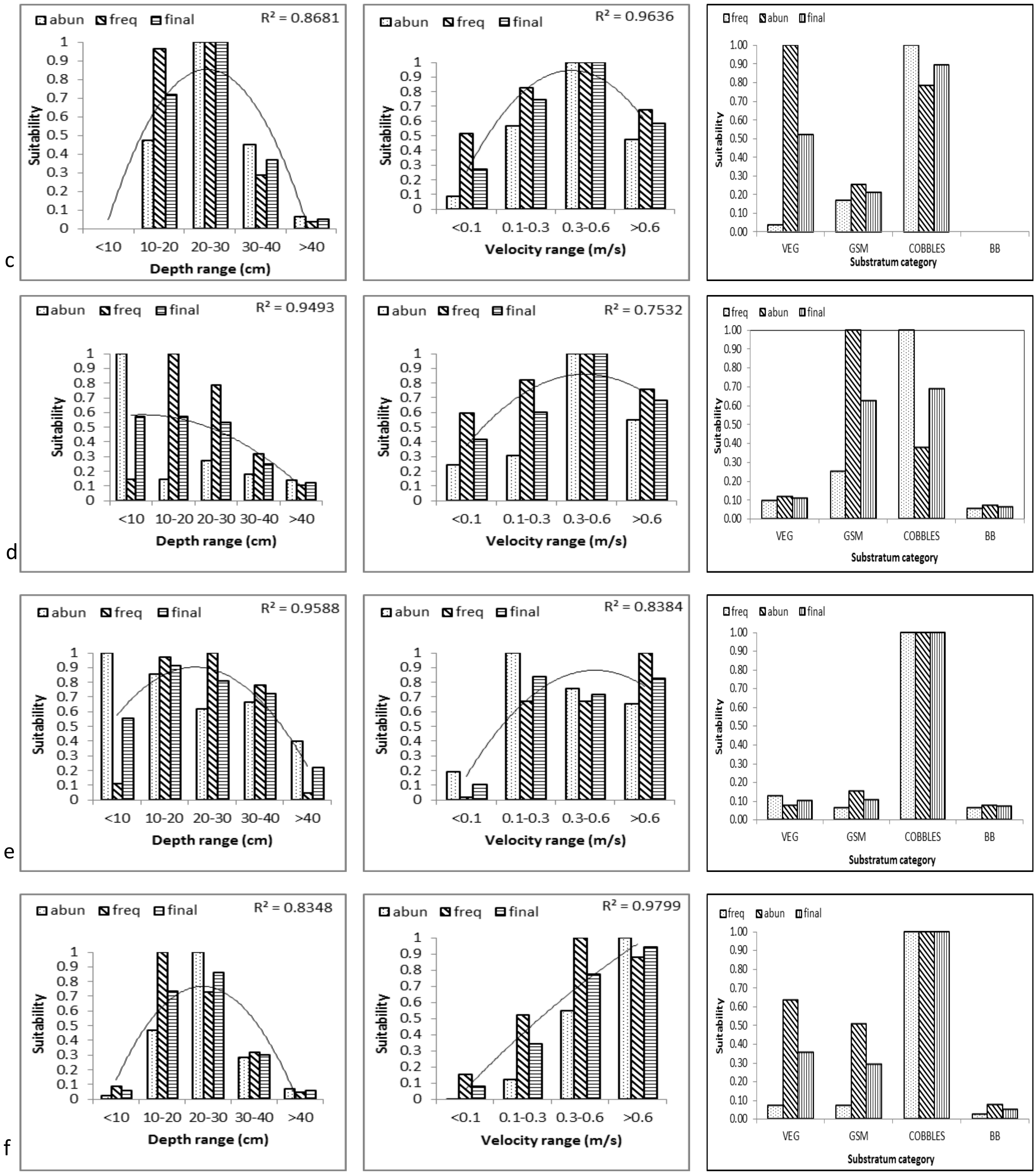

Figure 3.7 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Ephemeroptera (a) Baetidae, (b) Caenidae, (c) Heptageniidae, (d) Leptophlebiidae, (e) Teloganodidae, and (f) Tricorythidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

Baetidae did not show a clear preference for any specific velocity or substratum category, but showed a preference for water depths between 10 and 30 cm (Figure 3.7 a). Caenidae occurred mostly at depths of 10 - 30 cm and standing (<0.1 m/s) water in the GSM substratum (Figure 3.7 b). Heptageniidae also preferred the 10 - 30 cm depth range but preferred fast (0.3 - 0.6 m/s) water and a cobble substratum (Figure 3.7 c). Leptophlebiidae preferred shallow (<30 cm) fast flowing (0.3 - 0.6 m/s) water over GSM and cobbles (Figure 3.7 d). Teloganodidae preferred 10 - 40 cm deep fast water over cobbles but with no clear preference for velocity (Figure 3.7 e), while the Tricorythidae preferred 10 - 30 cm deep very fast flowing (>0.6 m/s) water over cobbles (Figure 3.7 f).

Table 3.3: Summary of the number of samples, sites and specimens for different Trichoptera families. No Habitat Suitability Curves were determined for the families indicated in bold typeface.

Family	No of Samples	No of sites	No of Specimens	R^2 Depth	Significance (f) Depth	R^2 Velocity	Significance (f) Velocity
Ecnomidae	17	16	27	0.9653	0.0347	0.3396	0.1053
Hydropsychidae	143	49	2488	0.7583	0.2417	0.9883	0.0358
Philopotamidae	31	22	81	0.6901	0.3099	1	0.0024
Polycentropodidae	**4**	**3**	**8**	N/A	N/A	N/A	N/A
Psychomyiidae	**2**	**2**	**3**	N/A	N/A	N/A	N/A
Calamoceratidae	**3**	**2**	**17**	N/A	N/A	N/A	N/A
Glossosomatidae	**4**	**1**	61	N/A	N/A	N/A	N/A
Hydroptilidae	70	32	369	0.2462	0.7538	0.7299	0.4077
Lepidostomatidae	**5**	**4**	**8**	N/A	N/A	N/A	N/A
Leptoceridae	78	35	629	0.5168	0.4832	0.999	0.1812
Sericostomatidae	10	6	**19**	0.8328	0.1672	0.8287	0.4983

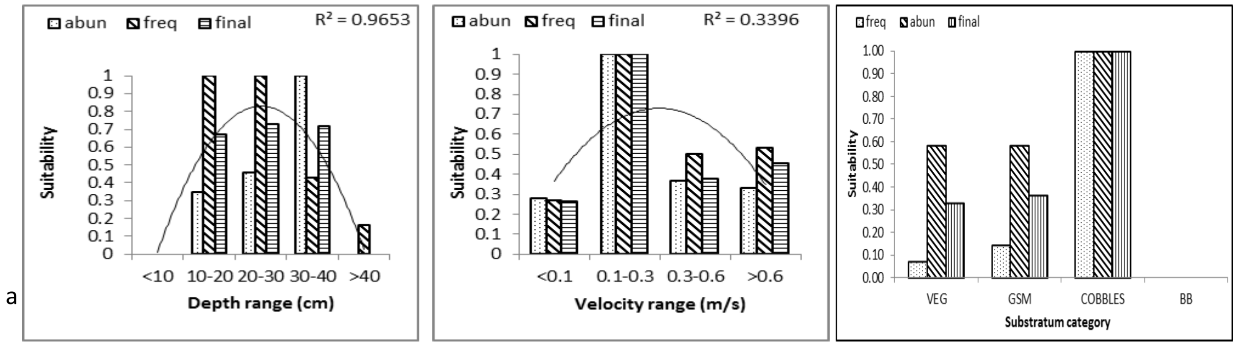

Figure 3.12: Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Trichoptera: (a) Ecnomidae, (b) Hydropsychidae, (c) Philopotamidae, (d), Hydroptilidae and (e) Leptoceridae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

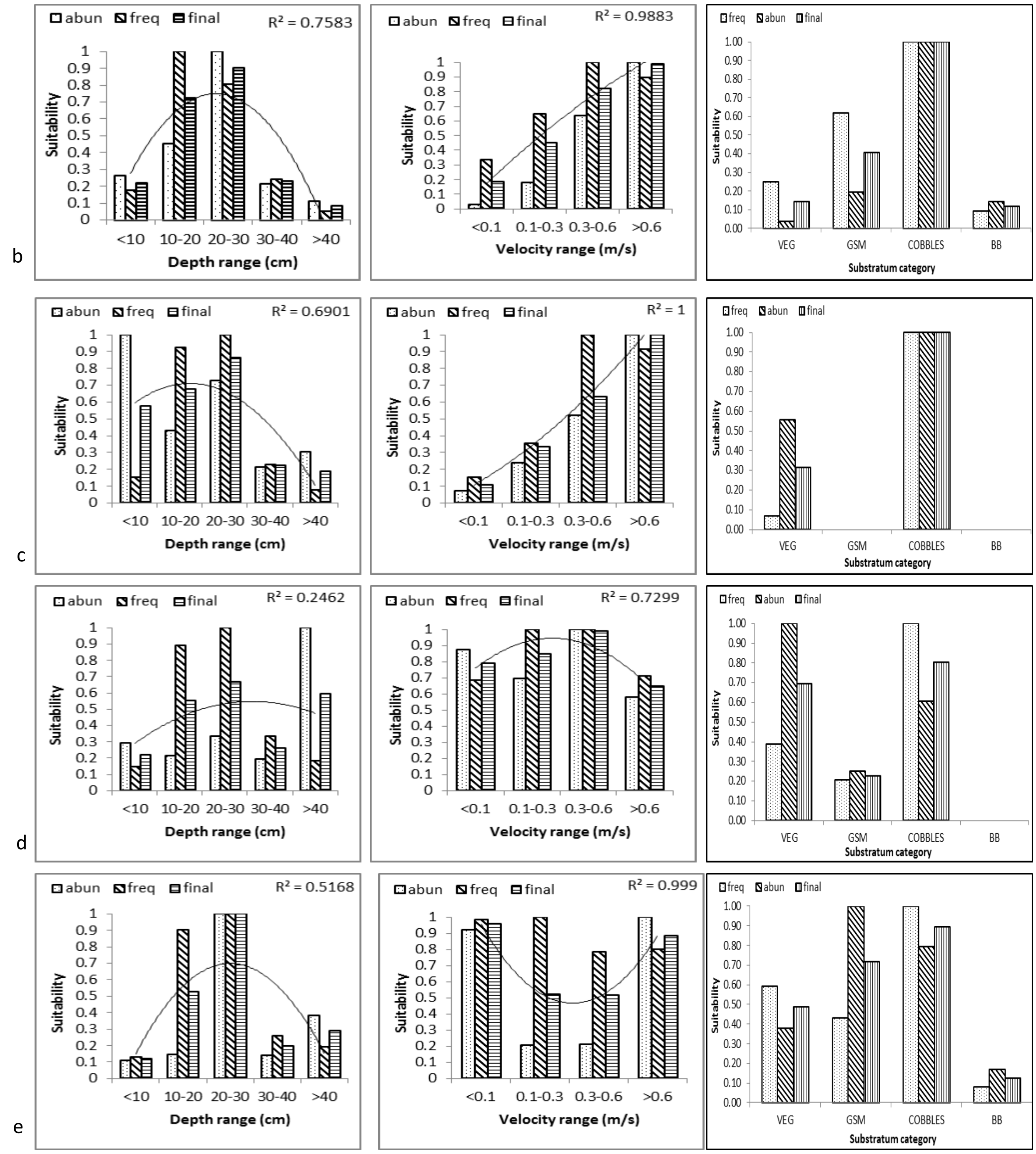

Figure 3.8 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Trichoptera: (a) Ecnomidae, (b) Hydropsychidae, (c) Philopotamidae, (d) Hydroptilidae and (e) Leptoceridae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

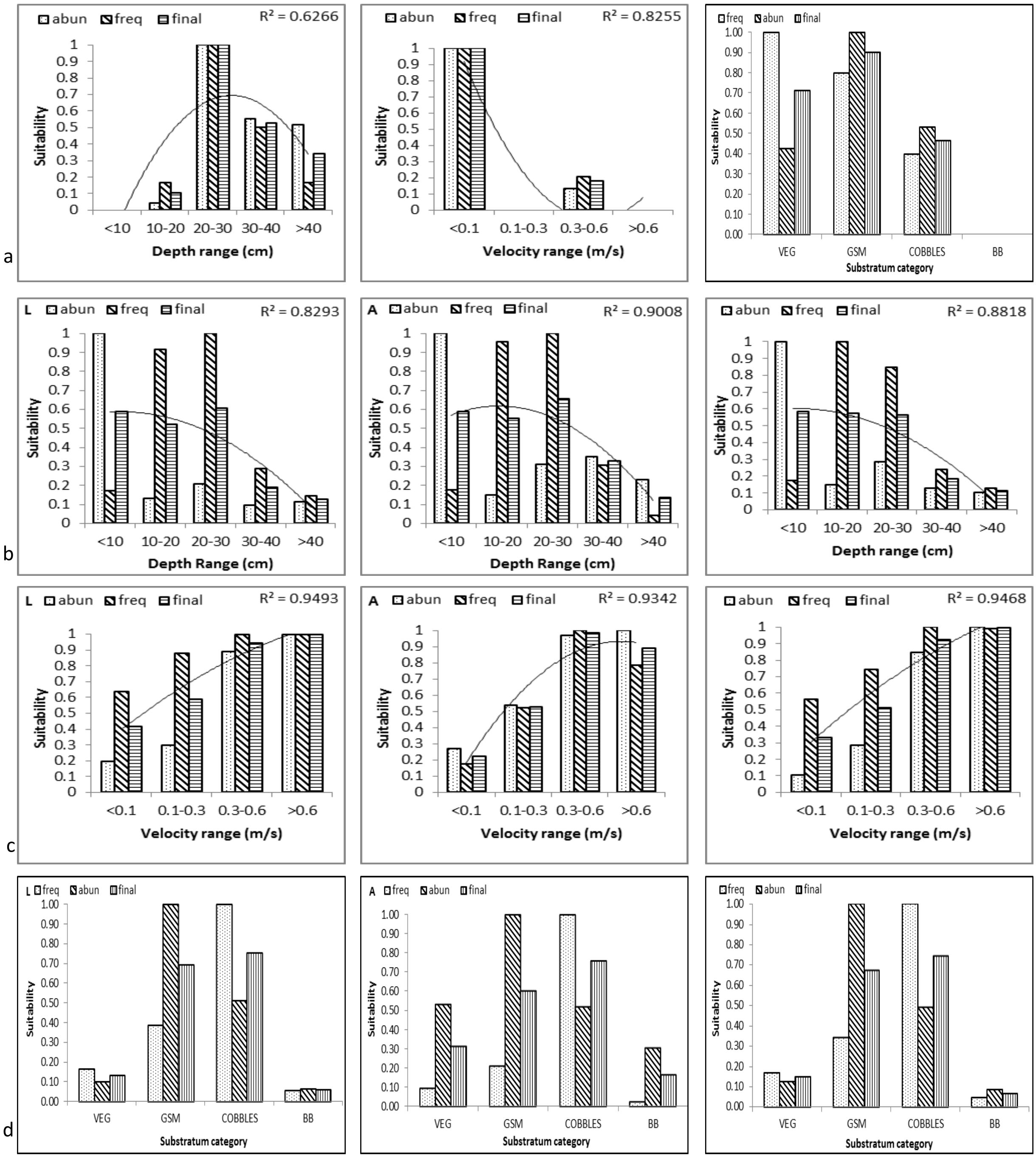

Figure 3.13: Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Coleoptera: (a) Dytiscidae, (b-d) Elmidae, (e-g) Gyrinidae, (h) Hydraenidae, (i) Hydrophilidae, (j) Scirtidae, and (k) Psephenidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

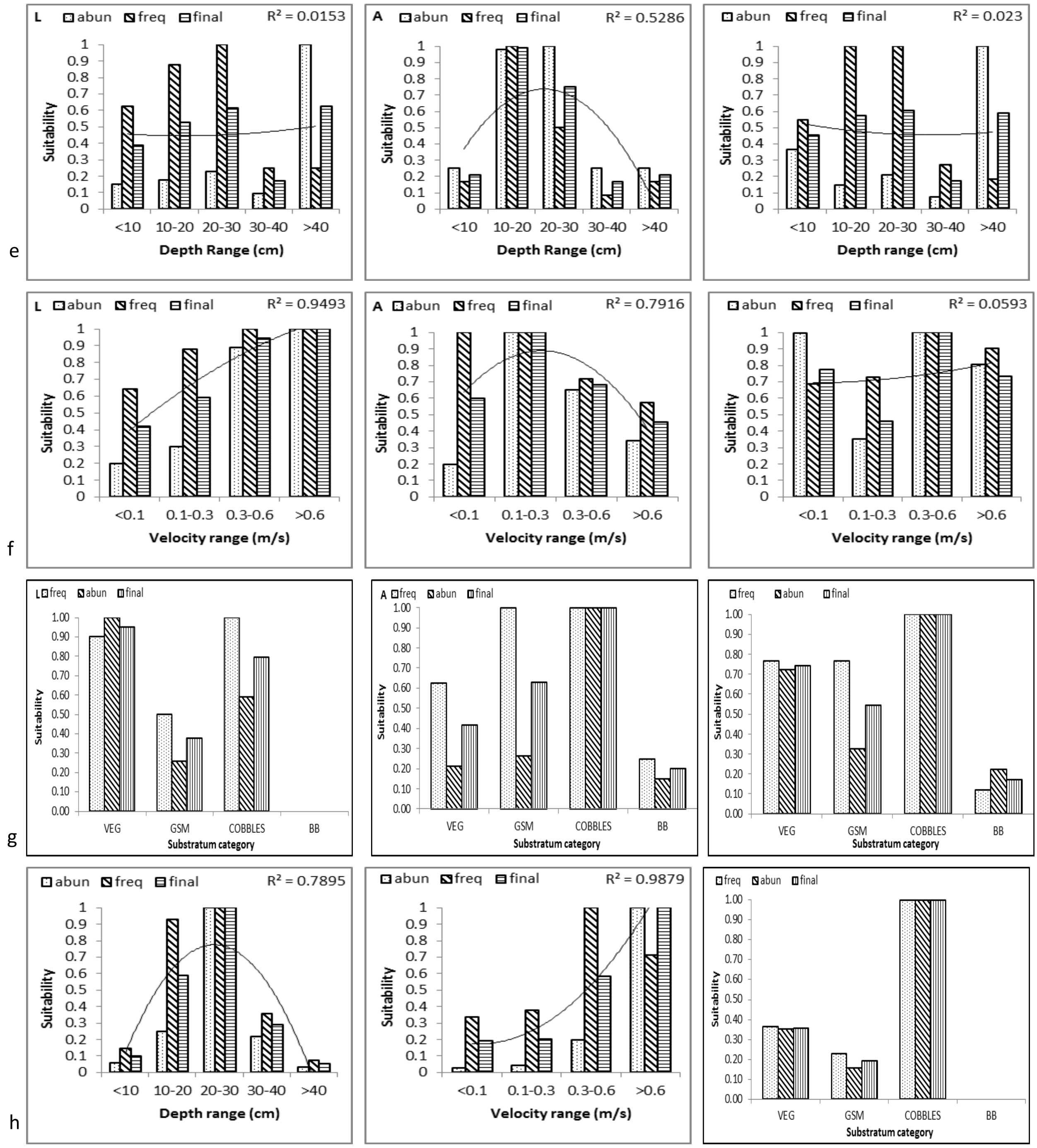

Figure 3.9 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Coleoptera: (a) Dytiscidae, (b-d) Elmidae, (e-g) Gyrinidae, (h) Hydraenidae, (i) Hydrophilidae, (j) Scirtidae, and (k) Psephenidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

Table 3.4: Summary of the number of samples, sites and specimens for different Coleoptera families. No Habitat Suitability Curves were determined for the families indicated in bold typeface. The larvae (L) and adults (Ad) were assessed separately as well when enough data were available.

Family	No of Samples	No of sites	No of Specimens	R^2 Depth	Significance (f) Depth	R^2 Velocity	Significance (f) Velocity
Dytiscidae L	**7**	**6**	**14**	N/A	N/A	N/A	N/A
Dytiscidae Ad	**4**	**4**	**13**	N/A	N/A	N/A	N/A
Dytiscidae	11	8	27	0.6266	0.3734	0.8255	0.1439
Elmidae L	87	37	1262	N/A	N/A	N/A	N/A
Elmidae Ad	57	29	222	N/A	N/A	N/A	N/A
Elmidae	100	38	1484	0.8818	0.1111	0.9468	0.1664
Gyrinidae L	24	20	63	N/A	N/A	N/A	N/A
Gyrinidae Ad	23	13	76	N/A	N/A	N/A	N/A
Gyrinidae	45	28	139	0.023	0.9770	0.0593	0.9561
Haliplidae L	**5**	**3**	**6**	N/A	N/A	N/A	N/A
Scirtidae L	22	13	91	0.1019	0.8981	0.9598	0.0971
Hydraenidae L	**1**	**1**	**4**	N/A	N/A	N/A	N/A
Hydraenidae Ad	35	21	223	N/A	N/A	N/A	N/A
Hydraenidae	35	21	227	0.7895	0.2105	0.9897	0.1076
Hydrophilidae L	**5**	**4**	**19**	N/A	N/A	N/A	N/A
Hydrophilidae Ad	13	11	35	N/A	N/A	N/A	N/A
Hydrophilidae	17	13	54	0.2927	0.70732	0.3216	0.824
Psephenidae	48	20	177	0.5171	0.4829	0.7216	0.4390
Ptilodactylidae	**3**	**3**	**6**	N/A	N/A	N/A	N/A

Not enough Dytiscidae were found to separate the adults and larvae (Table 3.4). Dytiscidae indicated a clear preference for 20 - 30 cm deep, standing (<0.1 m/s) water in vegetation and the GSM biotope (figure 3.9 a). Elmidae (larvae and adults) preferred the shallower (<30 cm) very fast (>0.6 m/s) flowing water although the adults also showed a preference for fast (0.3 - 0.6 m/s) flowing water (Figures 3.9 b – d). Both adult and larval Elmidae occurred in GSM and cobbles with a slightly higher preference for cobbles than GSM (Figure 3.9 d). However when the data for the adult and larval stages are combined, it seems as if the family have a greater preference for gravel than for cobbles (Figure 3.9 d). Larval Gyrinidae showed no preference for depth, while the adults occurred mostly in 10 - 30 cm deep water (Figure 3.9 e). Combined data for the adults and larvae showed no preference for depth (Figure 3.9 e). Larval Gyrinidae preferred very fast (>0.6 m/s) flowing water, whereas the adults occurred mostly in slow (0.1 - 0.3 m/s) flowing water (Figure 3.9 f). Combined data for the adults and larvae did not indicate a preference for any particular velocity category (Figure 3.9 f). Larval Gyrinidae occurred mostly in vegetation and cobbles, while the adults had a greater preference for GSM and cobbles (Figure 3.9 g). Combined larval and adult data indicated a strong preference for vegetation (Figure 3.9 g). Hydraenidae preferred 20-30 cm deep, very fast (>0.6 m/s) flowing water over cobbles (Figure 3.9 h). Hydrophilidae did not show a clear preference for a specific water depth or velocity category but a clear preference for vegetation was indicated (Figure 3.9 i). The Scirtidae did not show a clear preference for depth or velocity, but did exhibit a preference for vegetation (Figure 3.9 j). Psephenidae did not indicate a clear preference for water depth, but showed a slight preference for fast (0.3 - 0.6 m/s) flowing water and a clear preference for cobbles (Figure 3.9 k).

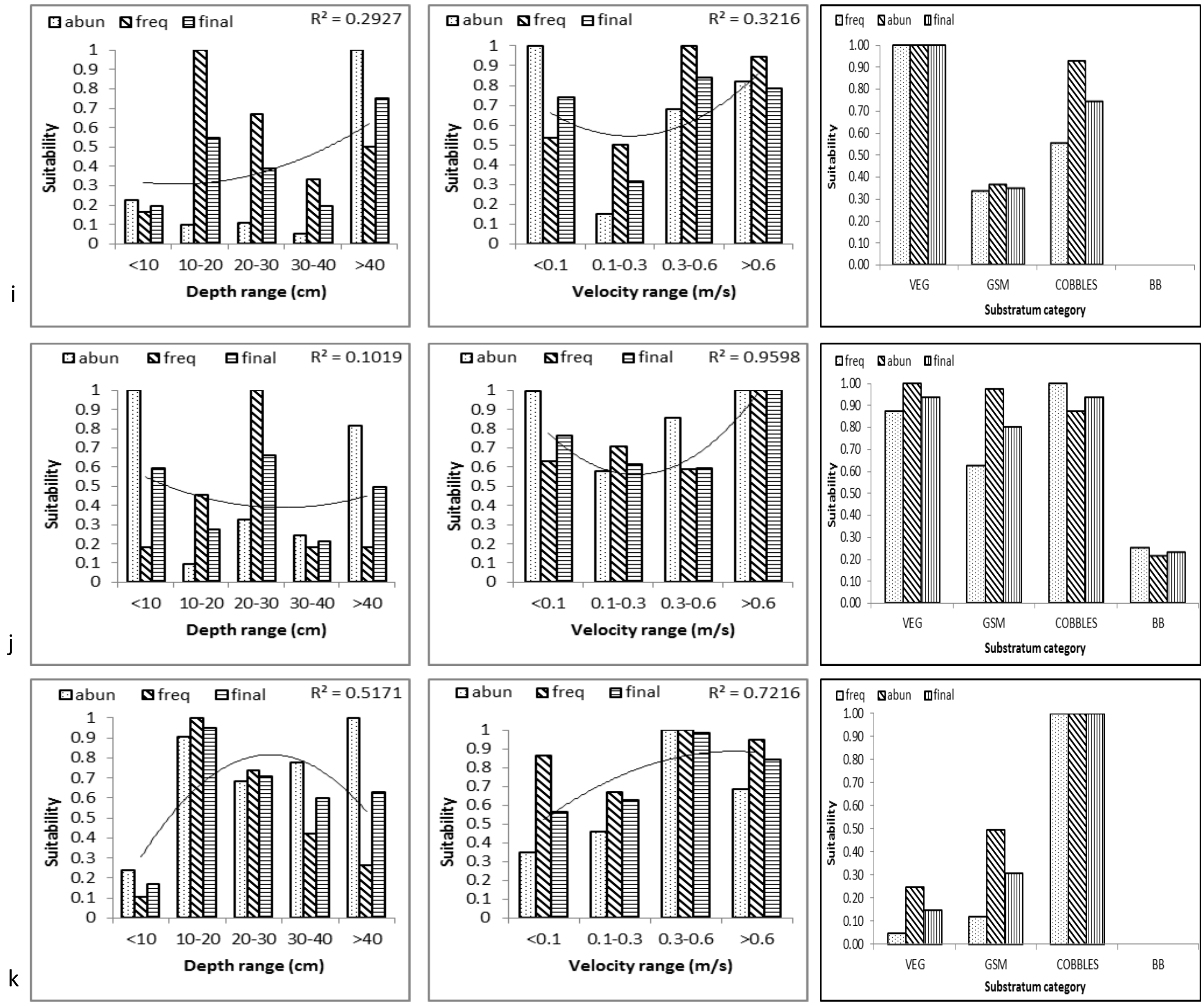

Figure 3.9 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Coleoptera: (a) Dytiscidae, (b-d) Elmidae, (e-g) Gyrinidae, (h) Hydraenidae, (i) Hydrophilidae, (j) Scirtidae, and (k) Psephenidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

The Athericidae preferred 10 - 20 cm deep, slow flowing (0.1 - 0.3 m/s) water over GSM and cobbles (Figure 3.10 a). Blephariceridae indicated a strong preference for 10 - 20 cm deep, very fast flowing (>0.6 m/s) water over cobbles (Figure 3.10 b). Ceratopogonidae (Figure 3.10 c) and Chironomidae (Figures 3.10 d-f) did not show a strong preference for water depth, velocity or substratum type. Empididae had a preference for 10 - 30 cm deep, fast flowing (0.3 - 0.6 m/s) water over cobbles (Figure 3.10 g). Simuliidae had a clear preference for 10 - 30 cm deep, very fast flowing (>0.6 m/s) water (Figures 3.10 h, i). The larval Simuliidae had a clear preference for the cobble biotope; while the pupae had similar preference values for vegetation and cobbles (Figure 3.10 j). Combined data for the larval and pupal Simuliid stages indicated a preference for cobbles (Figure 3.10 j). Tabanidae had a clear preference for 10 - 20 cm deep slow flowing (0.1 - 0.3 m/s) water over cobbles (Figure 3.10 k), whereas the Tipulidae preferred 10 - 30 cm deep water over GSM with no clear velocity preference (Figure 3.10 l).

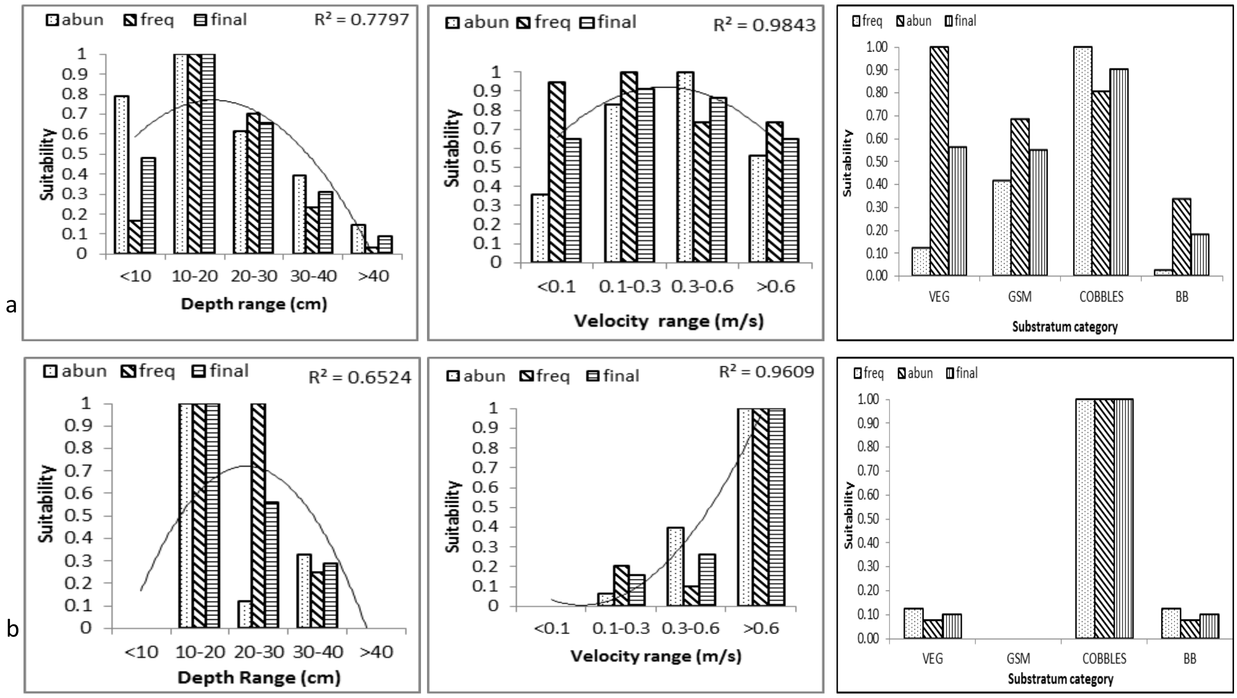

Figure 3.14: Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Diptera (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d-f) Chironomidae, (g) Empididae, (h-j) Simuliidae, (k) Tabanidae and (l) Tipulidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

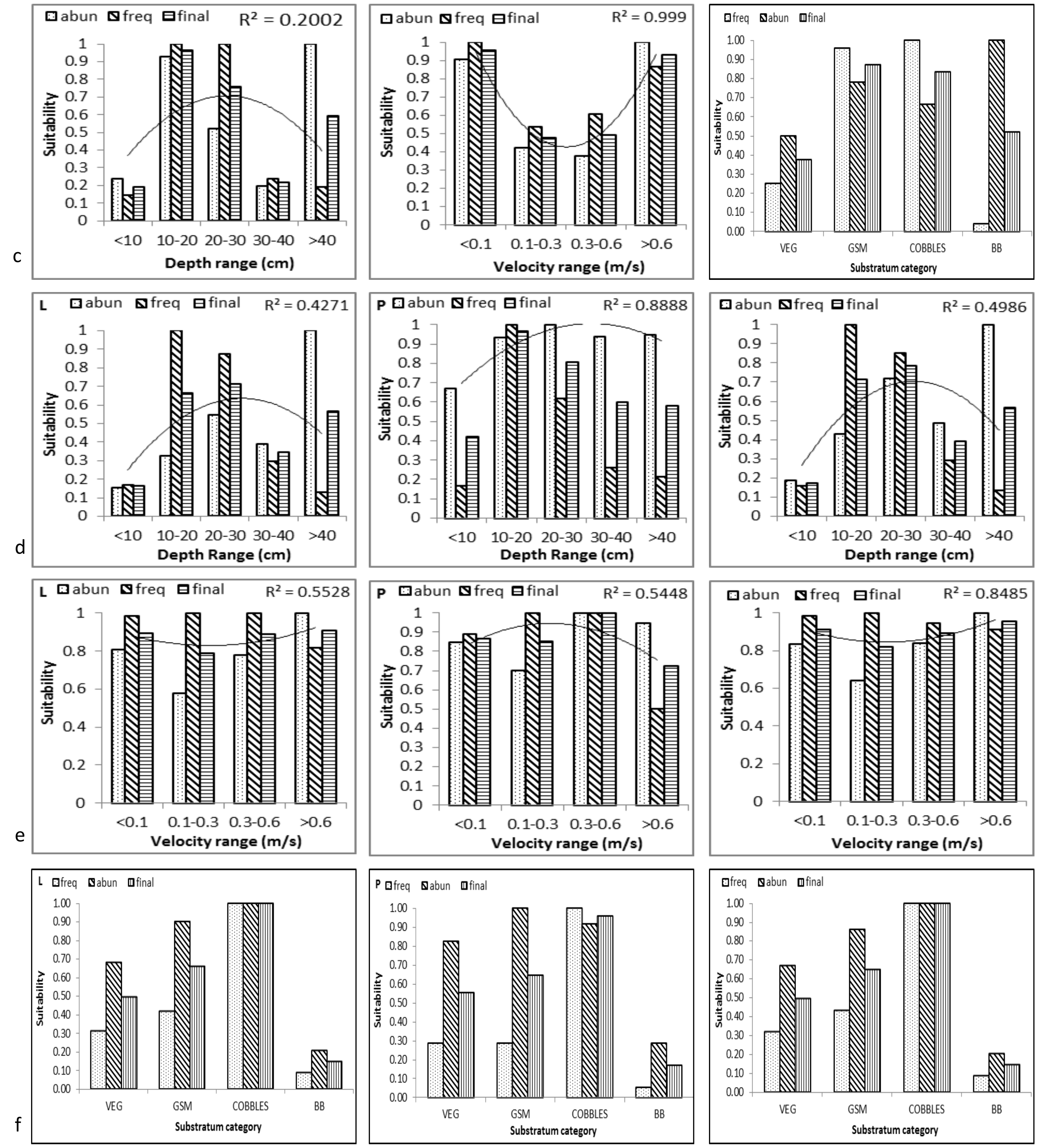

Figure 3.10 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Diptera (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d-f) Chironomidae, (g) Empididae, (h-j) Simuliidae, (k) Tabanidae and (l) Tipulidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

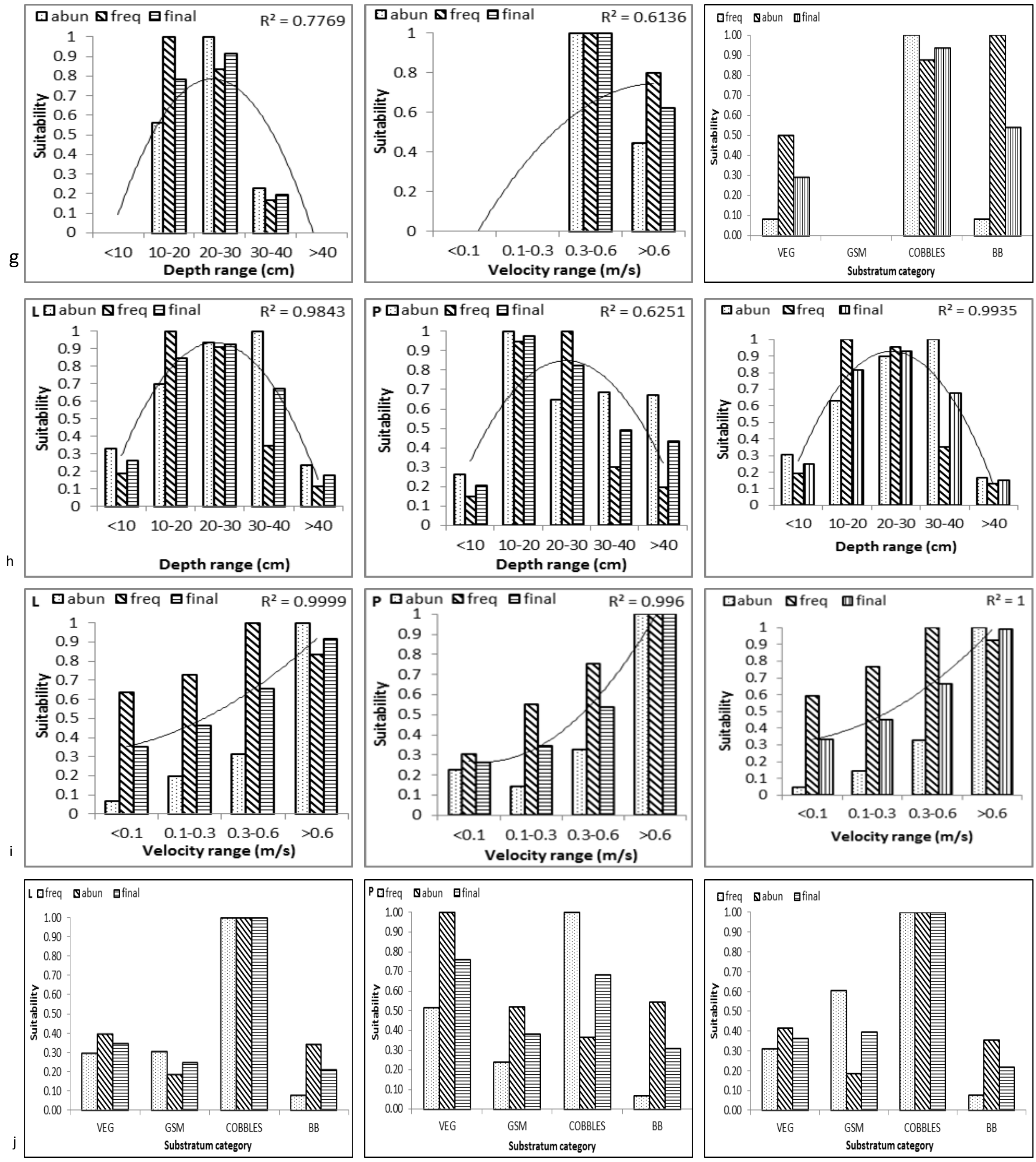

Figure 3.10 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Diptera (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d-f) Chironomidae, (g) Empididae, (h-j) Simuliidae, (k) Tabanidae and (l) Tipulidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

Table 3.5: Summary of the number of samples, sites and specimens for different Diptera families. No Habitat Suitability Curves were determined for the families indicated in bold typeface. The larvae (L) and pupae (P) were assessed separately as well when enough data were available.

Family	No of Samples	No of sites	No of Specimens	R^2 Depth	Significance (f) Depth	R^2 Velocity	Significance (f) Velocity
Athericidae	64	26	150	0.7797	0.2203	0.6808	0.1449
Blephariceridae L	**9**	**5**	59	N/A	N/A	N/A	N/A
Blephariceridae P	**2**	**2**	47	N/A	N/A	N/A	N/A
Blephariceridae	10	6	106	0.6531	0.3469	0.9609	0.1843
Ceratopogidae L	50	30	148	N/A	N/A	N/A	N/A
Ceratopogonidae P	10	9	42	N/A	N/A	N/A	N/A
Ceratopogonidae	54	31	190	0.2002	0.7998	0.999	0.1768
Chironomidae L	234	53	11709	N/A	N/A	N/A	N/A
Chironomidae P	96	42	339	N/A	N/A	N/A	N/A
Chironomidae	237	53	12048	0.4986	0.5014	0.8485	0.4751
Culicidae L	**1**	**1**	**1**	N/A	N/A	N/A	N/A
Culicidae P	**2**	**2**	**4**	N/A	N/A	N/A	N/A
Culicidae	**3**	**3**	**5**	N/A	N/A	N/A	N/A
Empididae L	12	9	**19**	N/A	N/A	N/A	N/A
Empididae P	**3**	**3**	**5**	N/A	N/A	N/A	N/A
Empididae	14	11	24	0.7769	0.223	0.6136	0.5130
Ephydridae L	**2**	**2**	**4**	N/A	N/A	N/A	N/A
Muscidae L	**1**	**1**	**2**	N/A	N/A	N/A	N/A
Psychodidae L	**2**	**1**	**3**	N/A	N/A	N/A	N/A
Simuliidae L	176	51	10388	N/A	N/A	N/A	N/A
Simuliidae P	53	27	251	N/A	N/A	N/A	N/A
Simuliidae	181	51	10639	0.9935	0.0065	1	0.0233
Tabanidae L	29	19	40	0.5757	0.4243	0.7333	0.6399
Tipulidae L	47	24	168	N/A	N/A	N/A	N/A
Tipulidae P	**2**	**2**	**2**	N/A	N/A	N/A	N/A
Tipulidae	47	24	170	0.8209	0.1791	0.6404	0.6791

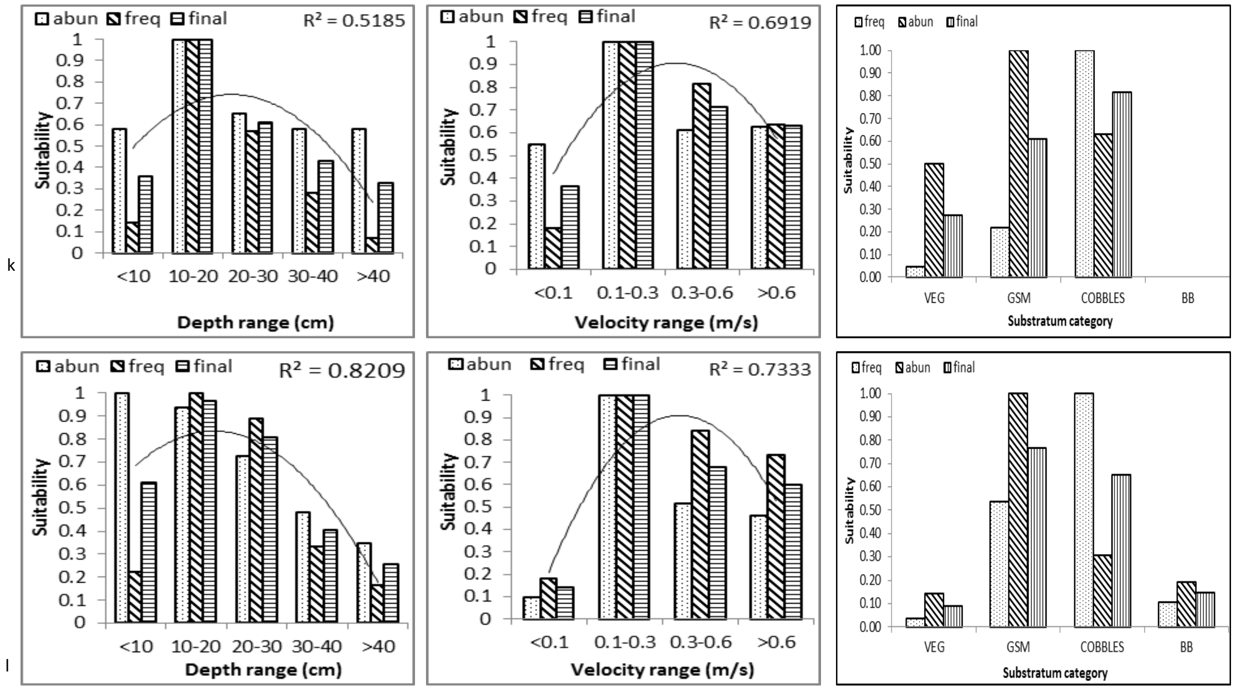

Figure 3.10 (continued): Habitat Suitability Curves based on depth (cm), velocity (m/s) and substratum category for Diptera (a) Athericidae, (b) Blephariceridae, (c) Ceratopogonidae, (d-f) Chironomidae, (g) Empididae, (h-j) Simuliidae, (k) Tabanidae and (l) Tipulidae. A second order polynomial regression was used to fit the data and where significant the R^2 value is provided. Vegetation: VEG; Gravel, Sand & Mud: GSM, Bedrock & Boulders:BB

3.4 Discussion

3.4.1 Ephemeroptera

Baetidae, as a family, do not present any clear preference for velocity or substratum (Figures 3.3 and 3.7 a), and seem to prefer water between 10 and 40 cm deep. According to other studies (Matthew 1968, Skoroszewski and de Moor 1999, Suter *et al*. 2002, Horta *et al*. 2009) Baetidae are a very diverse family with regards to the occurrence of individual species at different habitat types. The family includes vegetation specialists (*Pseudocloeon bellum, P. latum*), *Dabulamanzia indusii* preferring bedrock, *Cheleocloeon* excisum on sand, and numerous species living on cobbles and at all velocities and depths (Matthew 1968, Skoroszewski and de Moor 1999).

Caenidae clearly prefer standing water (<0.1 m/s) in the GSM biotope (Figure 3.3 and 3.7 b). Although the GAM indicates that Caenidae prefer either shallow (<10 cm) or deep (>40 cm) water, the HSC for Caenidae indicates a preference for 10 - 30 cm deep water. The reason for the apparent discrepancy could be related to the way the analyses were done. The HSC was constructed for different depth categories rather than a continuous range of depths. Matthew (1968) found seven *Caenis spp*. occurring in sand, cobbles and bedrock, whereas Suter *et al.* (2002) in Australia found Caenidae in 10 - 40 cm deep water over medium sized gravel out of current (<0.1 m/s).

Heptageniidae have a clear preference for 20 - 30 cm deep, fast flowing (0.3 - 0.6 m/s) water over cobbles (Figures 3.3 and 3.7). These preferences confirm the information in the literature. Matthew (1968) found that although *Compsoneuriella njalensis* occurred in vegetation in slow flowing (<0.2 m/s) water, *Afronurus spp*. occurred in cobbles-in-current (0.2 - 0.5 m/s) whereas Skoroszewski and de Moor (1999) found *Afronurus peringueyi* on cobbles in 10 - 20 cm deep water.

According to the information from this study, Leptophlebiidae have a preference for shallow (<30 cm deep), fast flowing (0.3 - 0.6 m/s) water over large gravels to small cobbles, although they also occur at other velocity categories and substratum types (Figures 3.3 and 3.7). Information from local and international literature indicates that different species of Leptophlebiidae can have differing habitat requirements. *Deleatidium spp*. in New Zealand did not demonstrate significant habitat preferences (Jowett *et al*. 1991), whereas *Acanthophlebia cruentata* preferred hard bottoms with slow to moderate flow (<0.4 m/s) in shallow (<30 cm) water (Collier and Quinn 2004). Locally, Matthew (1968) found that while *Euthraulus spp*. preferred cobbles in moderately flowing (0.2 - 0.55 m/s) water, *Adenophlebia spp*. preferred cobbles-out-of-current and in Lesotho Skoroszewski and de Moor (1999) found that *Euthraulus elegans* occurred on gravel and pebbles at very slow to moderately flowing (0.01 - 0.49 m/s) water and *Adenophlebia auriculata* on cobbles at velocities <2 m/s and at depths of 10 - 40 cm.

Teloganodidae seem to prefer shallow (<30 cm) flowing water over cobbles (Figures 3.3 and 3.7). There is limited and generalised information on teloganodid habitat preferences in the literature (McCafferty and Benstead 2002, Pereira-da-Conceicoa and Barber-James 2013) that does not contradict the preferences as determined in this study.

The Tricorythidae's habitat preference of shallow very fast flowing water over cobbles (Figures 3.3 and 3.7) is similar to those reported in the literature (Matthew 1968, Skoroszewski and de Moor 1999), although Matthew (1968) also found that a *Dicercomyzon sp*. preferred slow flowing (<0.2 m/s) water in vegetation.

3.4.2 Trichoptera

Not much information on the habitat preferences of Ecnomidae is available in the literature, however, the requirements as indicated in Figures 3.4 and 3.8 (shallow, slow flowing water over cobbles) coincides with information in the literature (Scott 1985, Picker *et al*. 2003, de Moor and Scott 2003, Morse 2004).

Hydropsychidae as a family prefer shallow (20 - 30 cm), very fast flowing (>0.6 m/s) water over cobbles (Figures 3.4 and 3.8), but different genera and species have slightly different requirements (Gore and Judy 1981, Orth and Maughan 1983, Skoroszewski and de Moor 1999, Picker *et al*. 2003, de Moor and Scott 2003).

The depth, velocity and substratum preferences for Philopotamidae (<30 cm deep, >0.6 m/s, large cobbles) as seen in Figures 3.4 and 3.8 largely coincides with information in the literature (Orth and Maughan 1983, Bonada *et al*. 2006, Horta *et al*. 2009). Picker *et al*. (2003) mentions that the family occurs mostly in fast flowing mountain streams while Scott (1985), de Moor and Scott (2003) and Bouchard (2004) all indicate that they occur in flowing water often in sheltered positions such as under submerged stones and logs.

Hydroptilidae as a family showed no clear habitat preference with regards to depth, velocity or substratum (Figures 3.4 and 3.8). Information from the literature is also inconclusive with Bouchard (2004) indicating that Hydroptilidae are found in all types of streams and lakes generally associated with submerged vegetation. Harby *et al.* (2007) mentions that *Hydroptila sp.* prefers slower water while Bonada *et al*. (2006) found that Hydroptilidae had no characteristic habitat type and Scott (1985) mentions that the caseless *Ugandatrichia* is modified for life in waterfalls and torrents.

Leptoceridae are a very diverse family with a highly variable range of habitats (Morse 2004). Although they are most common in standing waters according to Bouchard (2004), de Moor and Scott (2003) mentions that species with heavier (sand) cases live in faster flows. The information from the literature confirms the results from this study (Figures 3.4 and 3.8) that as a family the Leptoceridae have no clear velocity or substrate preference although they seem to prefer shallow (20 - 30 cm deep) water.

3.4.3 Coleoptera

The Dytiscidae are a very diverse family that occur in most freshwater bodies (Picker *et al.* 2003, Gioria 2014) including lentic and lotic systems (Biström 1985, Jäch 1998, Biström 2008) as well as temporary (Biström 1985) and brackish (Biström 2008) water. The results from this study (Figures 3.5 and 3.9) indicate a preference for shallow, lentic waters but no clear preference towards a specific substratum.

Elmidae are generally regarded as lotic (Penrith 1985a, Jäch 1998), but can be found in lentic water as well (Degani *et al*. 1993) as is also evident from Figure 3.9. Elmidae generally seem to prefer shallower water (Skoroszewski and de Moor 1999, Elliot 2008), but can occur on a variety of substrata including vegetation (Skoroszewski and de Moor 1999, Elliot 2008) sand and gravel (Nelson 2008a), although they are most often associated with cobbles (Orth and Maughan 1983, Penrith 1985a, Nelson 2008a, Nelson 2008b, Elliot 2008, Bereczki *et al*. 2012). The results from this study do not give a clear indication as to the substratum preference of Elmidae with both cobbles and GSM (Figure 3.9) seeming to provide good habitat. The reason for this apparent lack of habitat specificity could be related to different species preferring different habitats (Elliot 2008), larvae and adults preferring different habitats (Orth and Maughan 1983, Elliot 2008, Nelson 2008) as well as seasonal changes in habitat (Elliot 2008) as was found by Bereczki *et al.* (2012) with *Elmis maugetii* preferring cobbles in summer and pebbles in winter.

Gyrinidae are widespread cosmopolitan beetles that can inhabit both lotic and lentic (Jäch 1998, Stals 2008b) and even temporary (Stals 2008b) waters. Gyrinidae larvae and adults occur in different habitats (Figure 3.9) with the larvae preferring vegetation while the adults were mostly associated with GSM. In addition the adults occur on the water surface (Biström 1985, Picker *et al*. 2003, Stals 2008b) while larvae live at the bottom of pools (Picker *et al.* 2003) or crawl on submerged vegetation (Biström 1985).

Adult Hydraenidae are generally aquatic (Penrith 1985a, Jäch 1998) or psammophilous (Perkins 2008) and occur both in fast flowing, highly oxygenated mountain streams and in stagnant even saline pools (Penrith 1985a) while the semi-aquatic larvae tend to live in damp soil at the water's edge. Skoroszewski and de Moor (1999) found that Hydraenidae had a preference for shallow (<20 cm) flowing water (0.01 - 0.49 m/s) over cobbles whereas Degani *et al.* (1993) in northern Israel found that they preferred very fast flowing (>0.6 m/s) and deeper (30 – 60 cm) water. The apparent differences in habitat preference might be related to the species collected as the literature indicates a variety of habitats such as the underside of the surface film (Penrith 1985a), the edges of sandy or gravelly streams or vegetation at the margins of streams (Perkins 2008). Perkins (2008) also mentions that members of some genera are typically found in the stones-in-current biotope whereas others inhabit hygropetric habitats such as seepage areas or the splash zone at the margins of waterfalls.

As can be expected from such a diverse family as Hydrophilidae, there is no clear habitat preference on a family level (Jäch 1998, Stals and Endrödy-Younga 2008) apart from an apparent preference for vegetation with cobbles also inhabited (Figures 3.5 and 3.9). Information from the literature also indicates this family's diverse habitat preferences, with Picker *et al*. (2003) and Penrith (1985b) indicating the undersides of submerged stones providing habitat, whereas according to Stals and Endrödy-Younga (2008) they are encountered in almost any freshwater habitat with smaller adults running upside down under the water surface while others live in moss or hygropetric environments.

Scirtidae do not appear to have a preferred habitat apart from not really favouring bedrock (Figures 3.5 and 3.9). The limited information from the literature suggests that they are largely associated with water or damp terrestrial conditions (Endrödy-Younga 1985, Lawrence and Ślipiński 2013), they can occur in still-, running- or groundwater (Jäch 1998, Endrödy-Younga and Stals 2008c), generally in the shallows associated with marginal vegetation (Endrödy-Younga and Stals 2008c).

Larval Psephenidae feed on epilithic algae, mostly at night (Bouchard 2004, Shepard and Lee 2008) and seem to prefer shallow, fast to very fast flowing water over cobbles (Figures 3.5 and 3.9). Although Orth and Maughan (1983) found that *Psephenus herricki* prefers slower water as well, the literature generally suggests that they occur on rocks in fast flowing (Picker *et al.* 2003, Bouchard 2004, Shepard and Lee 2008) or running water (Jäch 1998).

3.4.4 Diptera

According to the literature (Barraclough and Londt 1985, Harrison *et al*. 2003a, Picker *et al*. 2003) the predatory larvae of Athericidae occur mostly in flowing streams. Figures 3.6 and 3.10 also indicate a preference for shallow, flowing water over cobbles although they also occurred in the GSM and vegetation samples. Bulánková and Duricková (2009) positively correlated the occurrence of *Atherix ibis* with stream order and warmer temperatures, whereas Glime (2015) found *A. variegata* mostly in moss habitat. In South Africa, Picker *et al*. (2003) mentions that in the southern Cape *Tricantha atranupta* clings to the underside of logs and causeways above fast-flowing streams whereas *Atrichops adamaster* prefers rocks in mountain streams.

Blephariceridid larvae and pupae occur on rock surfaces in the fastest flowing sections (Baraclough and Londt 1985, Harrison *et al.* 2003a, Harrison *et al*. 2003b, Picker *et al*. 2003) of cool, well-oxygenated unpolluted mountain streams (Picker *et al*. 2003) and can also exist on vertical cascades (Barraclough and Londt 1985) or the spray of waterfalls, with many species endemic to small areas. In South Africa, this family is generally restricted to the mountain ranges of the southern-, western-, and eastern Cape as well as the KwaZulu-Natal and Mpumalanga Drakensberg although they have also been found near the source of the Groot Marico River in the Northwest Province that is fed by cold, clean water from a dolomitic eye (Chapter 2). It is clear that this family prefers shallow, very fast flowing water over cobbles (Figures 3.6, 3.10), but the range is restricted by temperature. Although Figures 3.6 and 3.10 clearly indicate a preference for cobbles, personal observation over more than 20 years as well as information from the literature, indicates that this family also frequently occurs on larger sized substrata such as bedrock and boulders.

Ceratopogonidae are often missed in SASS5 samples due to their small size, the larvae, however, occur in virtually all aquatic or semi-aquatic habitats (De Meillon and Wirth 2003). According to Picker *et al*. (2003) the larvae develop in water bodies with a high organic content, while Barraclough and Londt (1985) state that they occur in the mud or sand on the margins of streams, ponds or lakes, with the pupae occurring in the same environments as the larvae (De Meillon and Wirth 2003). The Ceratopogonidae preferred GSM and cobble substrata but showed no clear preference for any velocity category (Figures 3.6, 3.10). The GAM results (Figure 3.6) indicated a preference for deeper water whereas the HSC (Figure 3.10) indicated a preference for shallow (10 – 20 cm) water. Closer examination of the results indicates that the family most frequently occurred at 10 – 20 cm deep water but that the highest abundances occurred in deep (>40 cm) water explaining the apparent contradiction in the results.

Chironomidae are a very diverse family whose larvae occur in almost all freshwater and even some intertidal habitats (Harrison 2003, Picker *et al*. 2003). Due to the high diversity and wide distribution of this family, one can expect that they might not exhibit strict preferences regarding the habitats in which they occur. Horta *et al*. (2009) found that the family preferred smaller substrata, Orth and Maughan (1983) found a preference for slow flow over cobbles, while in New Zealand, Jowett *et al*. (1991) found that they have a broad substratum preference and a preference for low to moderate (<0.75 m/s) velocities. From Figures 3.6, 3.10, 3.10 and 3.10 it is clear that apart from an apparent slight preference for cobbles the Chironomidae do not exhibit habitat preferences. Closer inspection of the literature, however, does indicate some preferences at a higher taxonomic resolution. The larvae of *Chironomus* are adapted to live in low oxygen conditions and are often found in mud and rotting leaves of stagnant waterbodies and are therefore common in standing or slow-flowing backwaters of heavily polluted streams (Picker *et al.* 2003). The Chironominae subfamily prefers warmer water and standing or slowly flowing water (Barraclough and Londt 1985).

The majority of empidid species are not aquatic (Barraclough and Londt 1985, Harrison *et al*. 2003a) but the aquatic larvae are normally associated with vegetation at the edges of streams and rivers (Picker *et al*. 2003). Although Ivković *et al*. (2012) and Čmrelec *et al*. (2013) in eastern Europe found that larval Empididae preferred faster flowing water over moss, gravel, pebbles and tufa, Figures 3.6 and 3.10 indicated a greater preference for fast flowing (0.3 - 0.6 m/s) 10 – 30 cm deep water over larger substrata (cobbles, boulders and bedrock). This apparent difference in substratum preference might be due to different species in South Africa and eastern Europe. Another contributing factor could be that only limited aquatic vegetation was available at the sites used for this study.

Simuliid larvae and pupae are attached to inorganic or organic substrata in flowing water (Barraclough and Londt 1985, de Moor 2003, Picker *et al*. 2003). Although Simuliidae tend to occur in fast flowing parts of streams and rivers (Barraclough and Londt 1985, Morin *et al*. 1986, Skoroszewski and de Moor 1999, de Moor 2003, Picker *et al*. 2003), there are species (*Simulium vorax*, *Simulium aureum*, *Stegopterna mutate* etc.) that are adapted to slower flowing water (Skoroszewski and de Moor 1999, Palmer and Craig 2000). Work done by Skoroszewski and de Moor (1999) in Lesotho, confirms the results from this study (Figures 3.6, 3.10). Although Simuliidae occur over a wide range of depths, velocity and substratum types, they generally prefer shallow (10 – 30 cm) very fast (>0.6 m/s) water over cobbles. Similar results have also been reported from other researchers (Orth and Maughan 1983, Morin *et al*. 1986, Degani *et al*. 1993, Malmqvist 1994, Palmer and Craig 2000, Pinto *et al*. 2014) in various other parts of the world.

Larval Tabanidae often occur in mud at the edges of waterbodies such as ponds, lakes and streams (Barraclough and Londt 1985, Picker *et al*. 2003, Bouchard 2004) although they can also occur in other habitats (Middlekauff and Lane 1980) such as sand or gravel in faster water (Bouchard 2004). The specimens found during this survey exhibited a preference for shallow (10 - 30cm) slow flowing water over cobbles (Figures 3.6, 3.10) rather than the more widely accepted mud in standing water (Middlekauff and Lane 1980, Barraclough and Londt 1985, Picker *et al*. 2003, Marshall and Courtney 2015).

Not all Tipulidae are aquatic (Barraclough and Londt 1985, Harrison *et al*. 2003c, Picker *et al.* 2003) and those that are aquatic occupy a variety of habitats (Gelhaus 2002) such as mats of algae or vegetation in shallow water (Picker *et al.* 2003, Bouchard 2004). Harrison *et al*. (2003c) mentions that the larvae of aquatic species usually occur in well aerated fast flowing water. According to Gelhaus (2002) they occur in shallow habitats such as ponds, streams, marshes and seasonally flooded forests where they feed on organic debris often in mud or sand or under rocks, but other species feed and live on mosses and algae in slow flowing water over rock faces or the splash zone of waterfalls. From Figures 3.6 and 3.10 one can see that this study indicates a preference for shallow, faster flowing water over GSM, with cobbles also a suitable substratum.

3.5 Conclusions

The hypothesis tested in this chapter was that the macroinvertebrate assemblage structure can be differentiated based on environmental factors such as substratum, depth, and velocity as well as physico-chemical parameters. The results indicated that this is true for certain taxa but not for others. It is also clear that certain environmental factors play a role in the distribution of certain taxa but not others (e.g. temperature is a determining factor for Blephariceridae, but not for Simuliidae). Interestingly enough depth was not a significant factor in determining the distribution of the insects under consideration. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on a combination of environmental factors and the null hypothesis that the macroinvertebrate assemblage structure cannot be differentiated based on environmental factors is therefore rejected.

The information obtained in this chapter provides a first step in setting habitat requirements for selected families of Ephemeroptera, Trichoptera, Coleoptera and Diptera. There is a need for more data on certain families such as Prosopistomatidae, Sericostomatidae, Glossosomatidae, Haliplidae, Ephydridae, Syrphidae and others not included in this chapter. The only way of obtaining data for these families is to actively target localities where they are known to occur. The structure of the sampling protocol actively excluded areas impacted by pollution, thereby excluding taxa such as Syrphidae that are known to occur in organically polluted water. The results from this chapter will be used to update the preference ratings in the Macroinvertebrate Response Assessment Index (Thirion 2007).

CHAPTER 4

UPDATE OF THE MACROINVERTEBRATE RESPONSE ASSESSMENT INDEX (MIRAI).

4.1 Introduction

The Macroinvertebrate Response Assessment Index (MIRAI) was developed as part of a suite of EcoStatus indices to be used in the Ecological Classification Process (Thirion 2007). These indices all follow the principle that biological responses (fish, macroinvertebrates and riparian vegetation) integrate the effect of the modification of the drivers (hydrology, geomorphology and physico-chemical conditions). This approach is based on rating the degree of change from natural on a scale of 0 (no change) to 5 (maximum change) for a variety of metrics. Each metric is also weighted in terms of its importance to determining the Ecological Category under natural conditions for the specific site or river reach under consideration. The EcoStatus models were developed following a Multi Criteria Decision Making Approach (MCDA). These models are used to determine an Ecological Category (EC) expressed in terms of A to F, where A represents a close to natural and F a critically modified condition. The detail and rationale for the EcoStatus models and the MIRAI are described in the EcoStatus manuals (Kleynhans and Louw 2007, Thirion 2007).

The main aim of the Ecological Classification process is to acquire a better understanding of the reasons for the system's deviation or Present Ecological State (PES) from the natural or reference condition. This information can then be used to derive desirable and attainable future objectives for the river. The Ecological Classification process forms an integral part of the Ecological Reserve Determination method and is also used in the River EcoStatus Monitoring Programme (REMP) to assess changes in biological responses in terms of the severity of biophysical changes. In contrast to the Ecological Reserve Determination process the REMP focuses primarily on the biological responses as an indicator of ecosystem condition. The REMP uses only a general assessment of the cause-and-effect relationship between the biological responses and the drivers. The EcoStatus of a site or river reach can be obtained using the River Data Integration (RIVDINT) system (Kleynhans and Thirion 2015a) or the Rapid

Habitat Assessment Method and Model (RHAMM) developed for use in both the REMP and Resource Directed Measures (RDM) processes (Kleynhans and Thirion 2015b). These methods have successfully been used in River EcoStatus monitoring in the Crocodile West Marico as well as the Inkomati Water Management Areas (Roux and Selepe 2013, Singh and Todd 2015).

Habitat functions as a temporally and spatially variable physical-, chemical-, and biological template within which freshwater macroinvertebrates can exist (Orth 1987, Poff and Ward 1990). The importance of physical habitat quantity and quality in determining the structure and composition of biotic communities have been demonstrated in numerous studies (e.g. Modde *et al.* 1991, Aadland 1993, Ebrahimnezhad and Harper 1997). In the context of this study, habitat can be defined as any combination of velocity, depth, substratum, physico-chemical characteristics and biological features that will provide the organism with its requirements for each life stage at a particular time and locality. These habitats can be grouped into specific macroinvertebrate biotopes such as stones, vegetation and gravel, sand and mud (GSM).

The distribution of a freshwater macroinvertebrate assemblage is set by the tolerance of the individuals in the population to an array of environmental factors. The distribution pattern of a freshwater macroinvertebrate taxon reflects the optimal overlap between habit and physical environmental conditions that comprise the habitat – substratum, flow and turbulence for example (Orth and Maughan 1983, Jowett *et al*. 1991, Mérigoux and Dolédec 2004, Brooks *et al.* 2005, Harby *et al.* 2007). The typically discontinuous, patchy distribution pattern of aquatic macroinvertebrates is ultimately the result of interplay between habitat, habit (behaviour pattern) and the availability of food resources (Cummins 1993). It is therefore essential that all habitat features are considered when evaluating the suitability of habitat for freshwater macroinvertebrates. The approach followed in assessing macroinvertebrate response to driver characteristics is based on a qualitative combination of information gained by a field survey, the available habitat as a result of driver conditions, and the traits of the macroinvertebrates present (Lamaroux *et al*. 2004 and Horta *et al*. 2009). The tolerance or preference values used in MIRAI were, to a large extent, based on limited information from the literature as well as personal experience (Thirion 2007) and it was recognised at the time that a more detailed study is necessary to improve these ratings. The main aim of this chapter was to update the preference/tolerance values of the aquatic macroinvertebrates in the MIRAI.

4.2 Materials and Methods

The Habitat Suitability Curves (HSCs) developed in the Chapter 3 were converted to values out of 5 (rounded to the nearest 0.5) to fit in with the system used in the suite of EcoStatus models. HSCs were also developed for taxa in the other macroinvertebrate orders and subsequently converted to values out of 5 and rounded to the nearest 0.5. Where no, or not enough, data were available, information from the literature was used to assign the preference values to each taxon. Teresa and Casatti (2013), in a study to develop habitat suitability criteria for Neotropical stream fish, suggested that HSC values greater than 0.7 can be regarded as preferred conditions. It was therefore decided to use preference values greater than 3.5 to indicate a strong preference for a certain habitat feature. This is in contrast to the original MIRAI where values of 3 and greater were used to indicate a preference for a specific metric (Thirion 2007). In instances where more than one metric had a preference value of greater than 3.5, the velocity or substratum category with the highest preference value was used to indicate the preferred conditions. In instances where more than one category had the same highest value, no preference for that metric was indicated. No changes were made to the physico-chemical (water quality) metric group as these ratings are based on the sensitivity values (QVs) used in the South African Scoring System (SASS) version 5 (Dickens and Graham 2002).

4.2.1 MIRAI v2

The Excel version of MIRAI v2 is included as an appendix. The basic principles and procedures for running MIRAI v2 is the same as what is described in the MIRAI manual (Thirion 2007). A brief summary of the model is given below.

The MIRAI consists of four different metric groups that measure the change of the present macroinvertebrate assemblage from the reference assemblage in terms of flow-, habitat- and physico-chemical (water quality) modification as well as alteration in system’s connectivity and seasonality. The change (increase or decrease) in terms of abundance and/or frequency of occurrence of the macroinvertebrate taxa with regards to the different metrics is measured on a scale from 0 (no change from reference) to 5 (extreme change from reference). Each metric (and metric group) is ranked and weighted according to its importance in determining the

Ecological Category (EC) of the macroinvertebrate assemblage. Each macroinvertebrate taxon is assigned a preference score ranging from no preference (0) to a very high preference (5) based on the HSCs developed in Chapter 3 as well as information from the literature. Preference scores greater than 3.5 are regarded as an indication of a strong preference for a certain metric category. The presence or absence, as well as the abundance and/or frequency of occurrence of taxa with a strong preference for a specific metric are evaluated. Any specific taxon should only be considered in one of the metric categories.

4.2.1.1 Flow modification

There are four different velocity categories within this metric group:

1. Very fast flowing water (>0.6 m/s)
2. Moderately fast flowing water (>0.3-0.6 m/s)
3. Slow flowing water (0.1-0.3 m/s)
4. Very slow flowing or standing water (<0.1 m/s).

4.2.1.2 Habitat modification

There are four habitat types within this metric group:

1. Cobbles include all hard surfaces larger than 16 mm. This biotope not only includes the traditional cobble size range, but also the smaller pebbles as well as the larger hard surfaces such as bedrock and boulders.
2. Vegetation includes all vegetation that can provide habitat for macroinvertebrates and does not distinguish between fringing- (marginal) and aquatic vegetation.
3. Gravel, Sand and Mud (GSM) is a combination of the smaller grain types and includes all grain sizes smaller than 16 mm.
4. Water Surface also includes the water column. Taxa included here do not have a preference for a specific substratum type as such.

4.2.1.3 Water Quality modification

There are four different groups within this metric group. These groups are based on the SASS5 weights (QVs).

1. High requirement for unmodified physico-chemical conditions: SASS5 weights 12-15
2. Moderate requirement for unmodified physico-chemical conditions: SASS5 weights 7-11
3. Low requirement for unmodified physico-chemical conditions: SASS5 weights 4-6
4. Very low requirement for unmodified physico-chemical conditions: SASS5 weights 1-3.

In addition to the standard set of metrics regarding the presence/absence and the abundance and/or frequency of occurrence of taxa, two additional metrics – the SASS5 score and the ASPT value, are included. Rating guidelines for these additional metrics remains the same as for MIRAI v1 (Thirion 2007).

4.2.1.4 System Connectivity and seasonality

The system connectivity metrics are only used where migratory taxa (e.g. the crustaceans Palaemonidae and *Varuna*) are expected to occur under reference conditions. In certain instances seasonal differences (e.g. Blephariceridae which is regarded as a winter taxon and Oligoneuridae which is regarded as a summer taxon) also come into play. This metric is also used where the natural seasonality of a river has been altered, usually due to unseasonal releases from impoundments.

4.2.1.5 Ecological Category (EC)

The four metric groups are combined to derive the macroinvertebrate Ecological Category (EC). The metric groups are ranked and weighted in a similar manner as the different metrics within each group. In cases where the system connectivity and seasonality metric group is not relevant it should be given a weight of 0%. The model then calculates the EC including boundary categories, based on the percentage of reference. These categories are presented in Table 4.1.

Table 4.1: Generic Ecological Integrity Categories (modified from Kleynhans 1996 and Kleynhans 1999)

ECOLOGICAL CATEGORY	GENERIC DESCRIPTION OF ECOLOGICAL CONDITIONS	ARBITRARY GUIDELINE SCORE (% OF MAXIMUM THEORETICAL TOTAL)
A	Unmodified/natural, close to natural or close too predevelopment conditions within the natural variability of the system drivers: hydrology, physico-chemical and geomorphology. The habitat template and biological components can be considered close to natural or to pre-development conditions. The resilience of the system has not been compromised.	>92 - 100
A/B	The system and its components are in a close to natural condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a B category.	>88 - ≤ 92
B	Largely natural with few modifications. A small change in the attributes of natural habitats and biota may have taken place in terms of frequencies of occurrence and abundance. Ecosystem functions and resilience are essentially unchanged.	>82 - ≤88
B/C	Close to largely natural most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a C category.	>78 - ≤82
C	Moderately modified. Loss and change of natural habitat and biota have occurred in terms of frequencies of occurrence and abundance. Basic ecosystem functions are still predominantly unchanged. The resilience of the system to recover from human impacts has not been lost and it is ability to recover to a moderately modified condition following disturbance has been maintained.	>62 - ≤78
C/D	The system is in a close to moderately modified condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of a D category.	>58 - ≤62
D	Largely modified. A large change or loss of natural habitat, biota and basic ecosystem functions have occurred. The resilience of the system to sustain this category has not been compromised and the ability to deliver ecological goods and services has been maintained.	>42 - ≤58
D/E	The system is in a close to largely modified condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of an E category. The resilience of the system is often under severe stress and may be lost permanently if adverse impacts continue.	>38 - ≤42
E	Seriously modified. The change in the natural habitat template, biota and basic ecosystem functions are extensive. Only resilient biota may survive and it is highly likely that invasive and problem (pest) species may dominate. The resilience of the system is severely compromised as is the capacity to provide ecological goods and services. However, geomorphological conditions are largely intact but extensive restoration may be required to improve the system's hydrology and physico-chemical conditions.	>20 - ≤38
E/F	The system is in a close to seriously modified condition most of the time. Conditions may rarely and temporarily decrease below the upper boundary of an F category. The resilience of the system is frequently under severe stress and may be lost permanently if adverse impacts continue.	>18 - ≤20
F	Critically/Extremely modified. Modifications have reached a critical level and the system has been modified completely with an almost complete change of the natural habitat template, biota and basic ecosystem functions. Ecological goods and services have largely been lost This is likely to include severe catchment changes as well as hydrological, physico-chemical and geomorphological changes. In the worst instances the basic ecosystem functions have been destroyed and the changes are irreversible. Restoration of the system to a synthetic but sustainable condition acceptable for human purposes and to limit downstream impacts is the only option.	≤18

4.2.2 Comparison of results from the two MIRAI models

Results from the HSCs as well as literature surveys indicated that no macroinvertebrate taxon had a strong preference for the Bedrock and Boulders metric (included in MIRAI v1) which was subsequently removed. The preference values in MIRAI v1 were then updated to reflect these new values. This new version (MIRAI v2) was then tested by running it for 44 sites as indicated in Figure 4.1 and comparing it to results obtained from the MIRAI v1. The results from the two different versions of MIRAI were compared using a one-way analysis of variance (ANOVA) and a linear regression analysis done using Excel 2010.

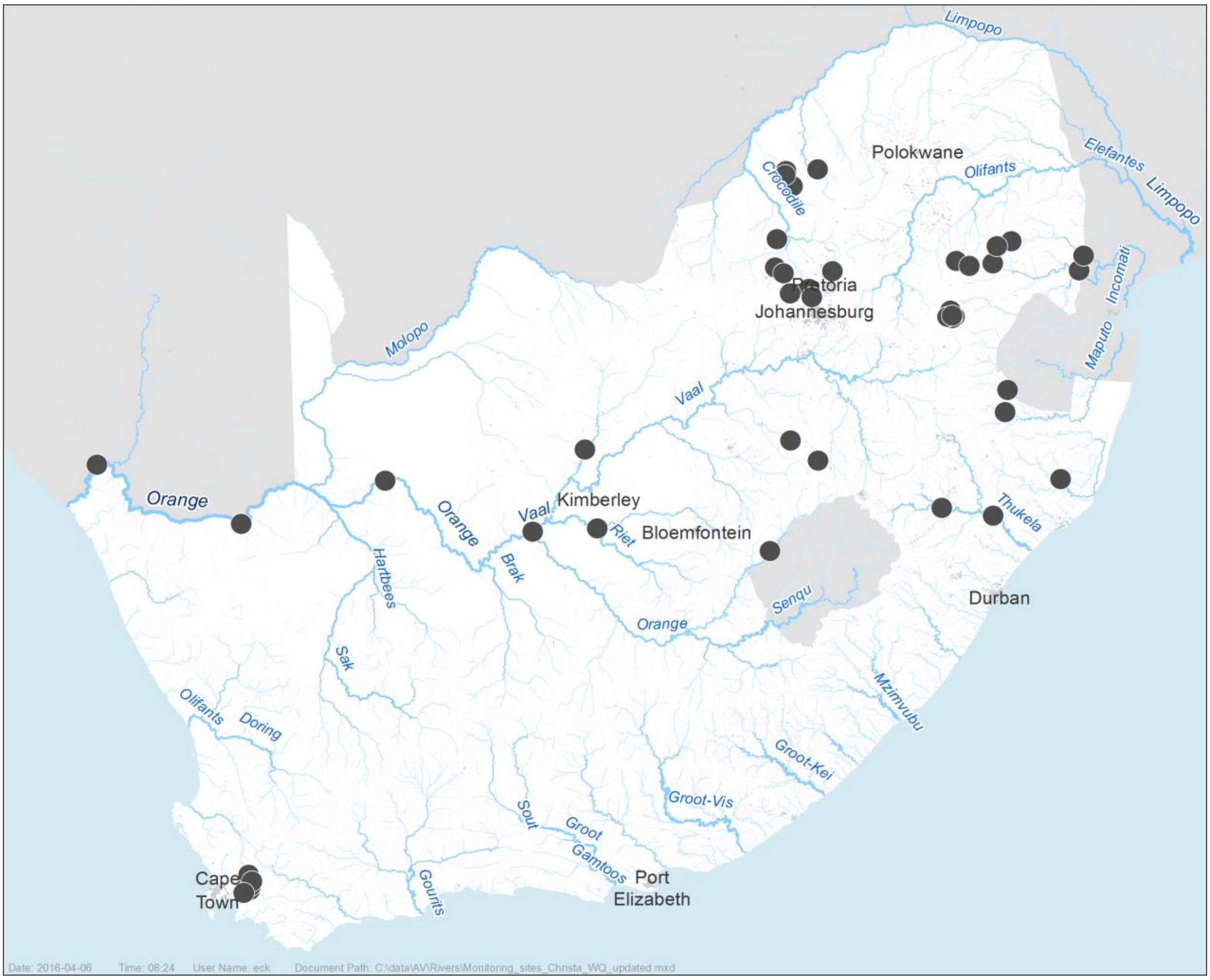

Figure 4.1: Map of South Africa indicating the 44 MIRAI test sites.

The 44 test sites covered seventeen Level I and 26 Level II Ecoregions (Kleynhans *et al.* 2005b), geomorphological zones ranging from Mountain Streams to Lowland Rivers (Rowntree *et al*. 2000) and Ecological Categories ranging from A/B to E/F (Table 4.1). The test sites can therefore be regarded as covering a wide enough range of conditions to test the MIRAI v2.

4.3 Results and Discussion

4.3.1 Preference ratings

The velocity preferences of 49 of the 95 MIRAI taxa, changed from the original MIRAI v1 to MIRAI v2. These changes are indicated in Table 4.2. The changes in velocity preference with the exception of Ephemeridae, Gomphidae, Naucoridae, Polycentropodidae and Haliplidae were small. The velocity preference ratings of taxa with no clear preference in MIRAI v1 were refined in MIRAI v2 while some taxa that had a preferred velocity category in the first version on closer inspection showed no clear preference for any particular velocity category in MIRAI v2. Some taxa such as Ceratopogonidae and Chironomidae had high preferences for a number of different velocity categories but no clear preference for any particular category. Ceratopogonidae showed a strong preference (4.5) for both standing and very fast flowing water whereas Chironomidae showed a strong preference (4.5) for all velocity categories. Chironomidae are a very diverse family and within the family have species with preferences for a different range of conditions. This makes the family a poor indicator of velocity changes although there might be species present that are more sensitive.

A third (34 taxa) of the 95 invertebrate taxa included in the MIRAI had a preference for standing (<0.1 m/s) water (Table 4.3). Although these taxa include both highly sensitive families such as the rare ephemerid burrowing mayfly (SASS5 QV=15), it also includes seven moderately sensitive (SASS5 QV 8-11) families and 11 families with a low requirement (SASS5 QV 4-7) for unmodified physico-chemical conditions. The other 15 taxa preferring standing water are tolerant to organic pollution such as the rat tailed maggots (Syrphidae) and muscid houseflies. Fifteen taxa each preferred slow flowing (0.1-0.3m/s) and moderately fast flowing (>0.3-0.6m/s) water, ranging from taxa with a very low requirement (SASS5 QV 1-3) for unmodified physico-chemical conditions through to highly sensitive taxa with a high requirement (SASS5 QV 12-15) for unmodified physico-chemical conditions. Only 14 taxa preferred very fast flowing (>0.6m/s) water. These taxa tend to more sensitive to physico-chemical changes. Six of the families with a preference for very fast flowing water are regarded as highly sensitive (SASS5 QV 12-15) to physico-chemical changes and five more are regarded as moderately sensitive (SASS5 QV 8-11) and the other three as having a low sensitivity (SASS5 QV 4-7) to physico-chemical changes. The 19 taxa with no specific requirement for any velocity category includes taxa such as the Amphipoda with little to no information regarding their preferences for different velocities apart from an apparent preference for flowing water (Griffiths and Stewart 2001), as well as the baetid mayflies and chironomid midges that include species occurring in standing and slow flowing water right through to other species that occur in very fast flowing water (Matthew 1968, Barber-James and Lugo-Ortiz 2003, Harrison 2003, Picker *et al*. 2003). Other taxa such as the commonly occurring potamonautid crabs and water mites (Hydracarina) occur at all velocity conditions with no obvious preference for a specific type of flow (Hart *et al*. 2001, Jansen van Rensburg and Day 2002). Although the filter feeding hydropsychid caddisflies and the blackflies (Simuliidae) are regarded as having a preference for very fast flowing water, both these families also include species that can occur in slower flowing water as well (Barraclough and Londt 1985, Scott 1985, de Moor 2003, de Moor and Scott 2003, Picker *et al*. 2003).

The study design did not allow for the inclusion of water surface or water column in the HSCs and as such preference ratings for the water surface and water column metric were based on information in the literature as well as practical experience and scientific judgment. The substratum preferences of 27 of the 95 MIRAI taxa, changed from MIRAI v1 to MIRAI v2. These changes are indicated in Table 4.4. In MIRAI v2, a substratum preference was determined for the Coelenterata (Pennak 1978, Rayner *et al*. 2002) and Lepidostomatidae (Scott 1985, de Moor and Scott 2003) that had no clear preference in the original MIRAI v1. The substratum preference of the five taxa that were indicated as having a preference for Bedrock and Boulders (Porifera, Polycentropodidae, Psychomyiidae, Petrothrincidae and Ancylidae) in MIRAI v1 was changed to a preference for Cobbles and the Bedrock/Boulder metric removed from the Habitat Modification metric group. A number of the Hemiptera in particular were indicated as having a preference for the water surface or water column. Although these families generally are found on the water surface, they are also often associated with the vegetation. In these instances the preference for those families were changed to Vegetation in MIRAI v2. Certain taxa such as Leptoceridae and Chironomidae, for instance, have a preference for more than one substratum type, depending on the species present (Barraclough and Londt 1985, de Moor and Scott 2003, Harrison 2003, Picker *et al*. 2003). The family Leptoceridae indicated a strong preference (4) for both GSM and vegetation and a slightly lower preference of 3.5 for cobbles. The majority of the Leptoceridae found in the vegetation had cases made of plant material whereas those in the GSM had cases made of sand grains.

Cobbles appear to be the preferred substratum for most taxa (Table 4.5). Although these 34 taxa includes the tolerant Turbellaria and Potamonautidae, and nine taxa with a low requirement for unmodified physico-chemical conditions, 15 of these taxa had a moderate- and ten a high requirement for unmodified physico-chemical conditions. Another 25 taxa preferred the vegetation, with nine of these taxa having a very low requirement for unmodified physico-chemical conditions and three having a high requirement for unmodified physico-chemical conditions. Five of the taxa preferring the vegetation biotope had a low requirement and another eight a moderate requirement for unmodified physico-chemical conditions. A total of 18 taxa preferred the GSM biotope.

The rare burrowing mayfly family, Ephemeridae preferring the GSM biotope has a high requirement for unmodified physico-chemical conditions (Crass 1947, Agnew 1985, Barber-James and Lugo-Ortiz 2003, Cahill 2004). Four taxa preferring the GSM biotope had a moderate-, eight a low- and five a very low requirement for unmodified physico-chemical conditions. Only six taxa had a preference for the water surface or water column. Most of these taxa had a low requirement for unmodified physico-chemical conditions with Dixidae (Barraclough and Londt 1985, Harrison *et al*. 2003a) having a high- and the Muscidae (Barraclough and Londt 1985, Harrison *et al*. 2003b, Picker *et al*. 2003) a very low requirement for unmodified physico-chemical conditions. Another 12 taxa showed no preference for any particular substratum type. These 12 taxa once again include the Baetidae and Chironomidae that on a family level do not show a preference although they contain species with highly specialised habitat requirements (Crass 1947, Matthew 1968, Agnew 1985, Barraclough and Londt 1985, Skoroszewski and de Moor 1999, Suter *et al*. 2002, Harrison 2003, Picker *et al*. 2003). Other families such as the Corixidae and Naucoridae (Calabrese 1985, Picker *et al*. 2003, Reavell 2003) do not seem to have a preference. Although the literature (Henning 2003) generally indicate that aquatic caterpillars of the Crambidae family occurs in the vegetation, they were also frequently encountered on cobbles and it was decided to not allocate a preferred substratum to them at this stage.

Table 4.2: Changes in velocity preference between the two MIRAI versions. Only taxa whose preference changed are indicated in the table. Preference values are indicted in brackets. Values indicated in bold denotes the preferred velocity category. Shaded cells indicate no clear preference.

Taxon	Velocity category (m/s) MIRAI v1	Velocity category (m/s) MIRAI v2
Porifera	No clear preference	0.1-0.3 (**4.5**)
Coelenterata	Slower water (2)	<0.1 (**4**)
Turbellaria	>0.6 (**4**)	0.3-0.6 (**4.5**)
Oligochaeta	Slower water (2)	<0.1 (**4.5**)
Hirudinea	Slower water (2)	0.1-0.3 (**4.5**)
Amphipoda	0.3-0.6 (**3**)	Flowing water (2.5)
Potamonautidae	0.3-0.6 (**3**)	No clear preference
Atyidae	Slower water (2)	<0.1 (**4**)
Palaemonidae	>0.6 (**3**)	Fast water (3)
Perlidae	>0.6 (**5**)	0.3-0.6 (**4**)
Ephemeridae	0.3-0.6 (**3**)	<0.1 (**4.5**)
Leptophlebiidae	<0.1 (**3**)	0.3-0.6 (**4.5**)
Polymitarcyidae	>0.6 (**3**)	<0.1 (**4.5**)
Teloganodidae	>0.6 (**4**)	0.3-0.6 (**4.5**)
Synlestidae	<0.1 (**3**)	Slower water (3)
Coenagrionidae	0.3-0.6 (**3**)	<0.1 (**4.5**)
Platycnemidae	0.1-0.3 (**3**)	Slow water (3)
Protoneuridae	0.1-0.3 (**3**)	Slow water (3)
Aeshnidae	Flowing water (2)	0.3-0.6 (**4**)
Corduliidae	0.1-0.3 (**3**)	<0.1 (**4**)
Gomphidae	0.3-06 (**3**)	<0.1 (**4.5**)
Crambidae	0.3-0.6 (**3**)	Faster water (3.5)
Corixidae	0.1-0.3 (**3**)	<0.1 (**4.5**)
Naucoridae	0.3-0.6 (**3**)	0.1-0.3 (**4**)
Ecnomidae	0.1-0.3 (**5**)	Flowing water (3.5)
Polycentropodidae	>0.6 (**4**)	0.1-0.3 (**4**)
Psychomyiidae/Xiphocentronidae	>0.6 (**3**)	0.3-0.6 (**4.5**)
Calamoceratidae	<0.1 (**4**)	0.1-0.3 (**4**)
Glossosomatidae	>0.6 (**4**)	0.3-0.6 (**4**)
Hydroptilidae	0.1-0.3 (**3**)	0.3-0.6 (**4.5**)
Leptoceridae	0.3-0.6 (**3**)	No clear preference (**4.5**)
Sericostomatidae	0.3-0.6 (**3**)	0.1-0.3 (**4**)
Elmidae	0.3-0.6 (**4**)	>0.6 (**4.5**)
Gyrinidae	>0.6 (**3**)	No clear preference (3.5)
Haliplidae	0.3-0.6 (**4**)	<0.1 (**4.5**)
Hydraenidae	0.3-0.6 (**3**)	>0.6 (**4**)
Limnichidae	0.1-0.3 (**3**)	<0.1 (**4**)
Psephenidae	>0.6 (**4**)	0.3-0.6 (**4.5**)
Athericidae	Flowing water (2)	0.1-0.3 (**4.5**)
Ceratopogonidae	>0.6 (**4**)	Standing and very fast flow (**4.5**)
Chironomidae	0.1-0.3 (**3**)	No clear preference (**4.5**)
Empididae	>0.6 (**4**)	0.3-0.6 (**4**)
Muscidae	>0.6 (**4**)	<0.1 (**4.5**)
Psychodidae	<0.1 (**3**)	0.1-0.3 (**4.5**)
Tipulidae	0.1-0.3 (**4**)	Flowing water (**4**)
Ancylidae	Flowing water (2)	0.3-0.6 (**4.5**)
Thiaridae	<0.1 (**3**)	0.1-0.3 (**4**)
Corbiculiidae	0.1-0.3 (**3**)	Slower water (**4**)
Sphaeridae	0.1-0.3 (**3**)	Slower water (**4.5**)

Table 4.3: Velocity preferences for different SASS5 taxa in the Macroinvertebrate Response Assessment Index version 2 (MIRAI v2)

Velocity preference per invertebrates				
<0.1m/s: Very slow or	0.1-0.3m/s: Slow	>0.3-0.6m/s: Moderately fast	>0.6 m/s: Very fast	No Preference
Coelenterata	Porifera	Turbellaria	Notonemouridae	Amphipoda
Oligochaeta	Hirudinea	Perlidae	Oligoneuridae	Potamonautidae
Atyidae	Calopterygidae	Heptageniidae	Prosopistomatidae	Palaemonidae
Caenidae	Chlorocyphidae	Leptophlebiidae	Tricorythidae	Hydracarina
Ephemeridae	Naucoridae	Teloganodidae	Hydropsychidae	Baetidae
Polymitarcyidae	Polycentropodidae	Aeshnidae	Philopotamidae	Synlestidae (Chlorolestidae)
Coenagrionidae	Calamoceratidae	Libellulidae	Hydrosalpingidae	Platycnemidae
Lestidae	Pisuliidae	Corydalidae	Petrothrincidae	Protoneuridae
Corduliidae	Sericostomatidae	Psychomyiidae/Xiphocentronidae	Elmidae	Crambidae (Pyralidae)
Gomphidae	Athericidae	Barbarochthonidae	Hydraenidae	Ecnomidae
Belostomatidae	Psychodidae	Glossosomatidae	Blephariceridae	Leptoceridae
Corixidae	Tabanidae	Hydroptilidae	Simuliidae	Gyrinidae
Gerridae	Thiaridae	Psepehnidae		Scirtidae (Helodidae)
Hydrometridae	Unionidae	Empididae		Hydrophilidae
Nepidae		Ancylidae		Ceratopogonidae
Notonectidae				Chironomidae
Pleidae				Tipulidae
Veliidae				Corbiculiidae
Sialidae				Sphaeridae
Dipseudopsidae				
Dytiscidae				
Haliplidae				
Limnichidae				
Culicidae				
Dixidae				
Ephydridae				
Muscidae				
Syrphidae				
Pomatiopsidae (Hydrobiidae)				
Lymnaeidae				
Physidae				
Planorbinae				
Viviparidae				

Table 4.4: Changes in substratum preference between the two MIRAI versions. Only taxa whose preference changed are indicated in the table. Preference values are indicted in brackets. Values indicated in bold denotes a high preference. Shaded cells indicate no clear preference

Taxon	Substratum MIRAI v1	Substratum MIRAI v2
Porifera	Bedrock/boulders (**3**)	Cobbles (**4.5**)
Coelenterata	Large substrata (2)	Vegetation (**4**)
Hirudinea	Cobbles (**4**)	Cobbles & GSM (**4**)
Amphipoda	GSM (**3**)	GSM (3.5)
Palaemonidae	Cobbles (**3**)	Cobbles & Vegetation (3)
Hydracarina	GSM (**3**)	Cobbles (3)
Platycnemidae	Vegetation (**3**)	Cobbles (**4**)
Crambidae	Vegetation (**3**)	Cobbles & Vegetation (3)
Corixidae	Water Surface (**4**)	GSM (3.5)
Naucoridae	Water Surface (**4**)	No clear preference (3.5)
Notonectidae	Water Surface (**4**)	Vegetation (**4**)
Polycentropodidae	Bedrock/boulders (**4**)	Cobbles (**4**)
Psychomyiidae/Xiphocentronidae	Bedrock/boulders (**4**)	Cobbles (**4.5**)
Barbarochthonidae	Cobbles (**3**)	Vegetation (**4**)
Calamoceratidae	GSM (**3**)	Vegetation (**4.5**)
Lepidostomatidae	No clear Preference (2)	Vegetation (**4**)
Petrothrincidae	Bedrock & Boulders (**4**)	Cobbles (**4**)
Pisuliidae	Cobbles (**3**)	Vegetation (**4**)
Sericostomatidae	Cobbles (**3**)	Vegetation (**4**)
Haliplidae	Vegetation (**4**)	Cobbles (**4**)
Hydraenidae	Vegetation (**3**)	Cobbles (**4**)
Ceratopogonidae	Cobbles (**3**)	GSM (**4**)
Culicidae	Water Surface (**5**)	Vegetation (**4**)
Psychodidae	Water Surface (**3**)	No clear preference (3.5)
Tabanidae	GSM (**3**)	Cobbles (**4**)
Ancylidae	Bedrock & Boulders (**3**)	Cobbles (**4**)
Thiaridae	Vegetation (**3**)	GSM (**4**)
Viviparidae	Vegetation (**3**)	GSM (**4**)

Table 4.5: Substratum preferences for different SASS5 taxa in the Macroinvertebrate Response Assessment Index version 2 (MIRAI v2)

Cobbles	Vegetation	Gravel Sand and Mud	Water Surface	No Preference
Porifera	Coelenterata	Oligochaeta	Gerridae	Hirudinea
Turbellaria	Atyidae	Caenidae	Hydrometridae	Amphipoda
Potamonautidae	Calopterygidae	Ephemeridae	Veliidae	Palaemonidae
Notonemouridae	Synlestidae (Chlorolestidae)	Polymitarcyidae	Gyrinidae	Hydracarina
Perlidae	Coenagrionidae	Corduliidae	Dixidae	Baetidae
Heptageniidae	Lestidae	Sialidae	Muscidae	Crambidae (Pyralidae)
Oligoneuridae	Protoneuridae	Dipseudopsidae		Corixidae
Prosopistomatidae	Belostomatidae	Limnichidae		Naucoridae
Teloganodidae	Nepidae	Ceratopogonidae		Leptoceridae
Tricorythidae	Notonectidae	Ephydridae		Psychodidae
Chlorocyphidae	Pleidae	Syrphidae		Hydrobiidae
Platycnemidae	Barbarochthonidae	Tipulidae		
Aeshnidae	Calamoceratidae	Viviparidae		
Libellulidae	Hydroptilidae	Corbiculiidae		
Corydalidae	Lepidostomatidae	Sphaeridae		
Ecnomidae	Pisuliidae	Unionidae		
Hydropsychidae	Sericostomatidae			
Philopotamidae	Dytiscidae			
Polycentropodidae	Scirtidae (Helodidae)			
Psychomyiidae/Xiphocentronidae	Hydrophilidae			
Glossosomatidae	Culicidae			
Hydrosalpingidae	Bulinae			
Petrothrincidae	Lymnaeidae			
Elmidae	Physidae			
Haliplidae	Planorbinae			
Hydraenidae				
Psepehnidae				
Athericidae				
Blephariceridae				
Empididae				
Simuliidae				
Tabanidae				
Ancylidae				

4.3.2 Statistical analysis

A comparison between the MIRAI v1 and MIRAI v2 index values are presented in Table 4.6. A one way ANOVA done on the results showed no significant difference between the two versions of MIRAI or the Velocity (Flow modification) and Substratum (Habitat modification) metric group results ($p>0.1$). The regression analyses results presented in Table 4.7 indicates that there is a good correlation ($R2>0.9$) between the two versions of MIRAI. The regressions are also presented graphically in Figure 4.2. As can be seen from Figure 4.2, there is more variation in the velocity and habitat modification metrics than in the overall MIRAI score when comparing the two versions of MIRAI.

Table 4.6: Comparison of the scores and categories based on the MIRAI v1 and v2 for 44 sites representing different ecoregions, geomorphological zones and levels of impact.

Site	**MIRAI v1**		**MIRAI v2**		**Percentage Change**
	Index Score	**Category**	**Index**	**Category**	
Mothlabatsi	88.2	AB	88.8	AB	0.6
Mamba	79.9	BC	80.7	BC	0.8
BM1	79.7	BC	81.9	BC	2.2
Pongola upper	78.8	BC	81.5	BC	2.7
X3SABI-LUNSK	81.6	BC	81.5	BC	0.1
Assegaai	87.3	B	87.2	B	0.1
Matlabas zyn kloof	86.6	B	89.4	AB	2.8
Mokolo 1B	79.1	BC	81.7	BC	2.6
A2MAGA-HARTE	75.8	C	74.5	C	1.3
A2CROC-HARTB	43.3	D	46.8	D	3.5
A2STER-MAMOG	51.6	D	57.3	D	5.7
A2HEX-ROOIW	54	D	56.7	D	2.7
A2PIEN-BUFFE	66.3	C	65.9	C	0.4
X21A-00930	85.6	B	87.3	B	1.7
A2APIE-VASTF	22.5	E	19.1	EF	3.4
X2AB-00962	87.4	B	87.9	B	0.5
X3SABI-OLIFA	78.6	BC	80.8	BC	2.2
X2HOUT-BRIDG	86.1	B	87.4	B	1.3
A2JUKS-DIENR	39.4	DE	34.1	E	5.3
BM MID	60.3	CD	58.3	CD	2
BM UP	76.3	C	77.1	C	0.8
BM ET	69.2	C	71.4	C	2.2
BM WT	53.2	D	56.3	D	3.1
C6VALS-LINDL	43.3	D	41.6	DE	1.7
BM LOWER	69.4	C	69.1	C	0.3
X2CROC-NGONG	66.3	C	70.7	C	4.4
X3SABI-BORDE	79.5	BC	78.2	BC	1.3
White Mfolozi	80.4	BC	80.6	BC	0.2
THEPS5	79.4	BC	82.1	B	2.7
THEPS4	82.2	B	80.1	BC	2.1
C6VALS-BETLE	54	D	51	D	3
OSAEH15.6 LHDA	41.5	DE	39.7	DE	1.8
G2EERS-JONKE	82.5	B	83.8	B	1.3
G1BERG-BRBM1	81.5	BC	82.4	B	0.9
G1OLIF-ABRID	84.5	B	86.4	B	1.9
G1BERG-BRBM2	77.3	C	77.7	C	0.4
G1HUGO-DEKKE	74.5	C	77.5	C	3
G1FRAN-LAPRO	56.7	D	55.6	D	1.1
OSAEH26.1 DOUGLAS	57.1	D	57.5	D	0.4
OSAEH26.17 GIFKLOOF	68.6	C	70.1	C	1.5
OSAEH28.1 PELLA	72.6	C	73.8	C	1.2
OSAEH 28.5 RICHTERS	83.3	B	82.5	B	0.8
C5RIET-JACOB	59.4	CD	58.4	CD	1
C5MODD-MODDE	51.4	D	50.9	D	0.5

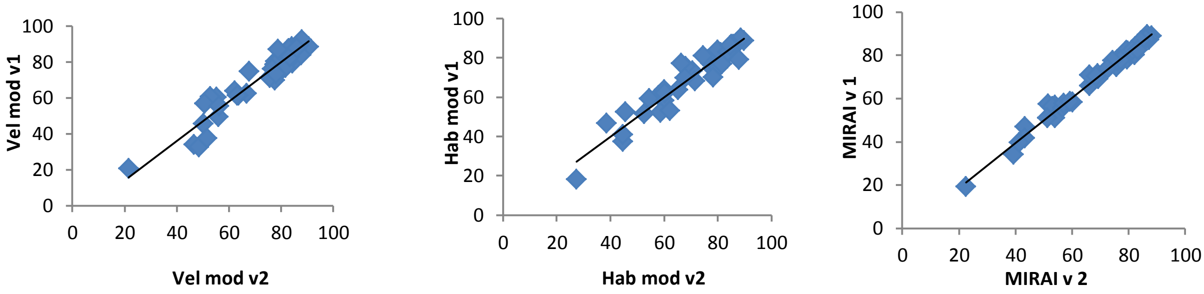

Figure 4.2: Scatterplots of the velocity modification, Habitat modification and MIRAI scores for the two versions of MIRAI for 44 test sites. The line indicating the best fit.

The changes to the velocity and substratum preference ratings in the MIRAI model resulted in only small changes to the total MIRAI score. The MIRAI category generally remained the same or at most changed by half a category. The largest percentage change was 5.7% at the Sterkstroom (Site A2STER-MAMOG) that remained in a D Category, and 5.3% at the Jukskei River (Site A2JUKS-DIENR) that changed from a DE category to an E category for MIRAI v2 (Table 4.6). The relatively small changes in MIRAI, together with the high correlation values (>0.9) means that the results from the two versions should be comparable and no adjustments will be needed to the results obtained from the original MIRAIs. The impact of the larger changes to the Flow modification and Habitat modification metric groups will however need to be investigated.

Table 4.7: Summary of regression analyses between MIRAI v1 and MIRAI v2 as well as the velocity and substratum metric groups.

Regression Statistics	Multiple R	R^2	Adjusted R^2	Standard Error
MIRAI	0.99245	0.984957	0.984599	1.98617
Velocity	0.956733	0.915339	0.913323	4.566874
substratum	0.958779	0.919257	0.917334	4.478539

4.4 Conclusions

Ever since the MIRAI was originally developed, it was recognised that the preference ratings for velocity and substratum types needed to be updated based on real data, rather than just a combination of very limited information from the literature and personal experience. Although this study only partly succeeded in gathering field-based data on the habitat requirements of the riverine macroinvertebrate taxa it has been a first step in improving the preference ratings used in the MIRAI. This study should be broadened to include taxa not yet studied, and redesigned to include the water surface and water column as a “substratum” type for macroinvertebrates. This will enable one to distinguish between the use of the water surface, in particular, and the marginal vegetation as a habitat for certain invertebrate taxa.

The following hypothesis was tested: The different habitat requirements of the invertebrate taxa in terms of velocity and substratum type can be used to refine the macroinvertebrate taxa’s preference ratings in the Macroinvertebrate Assessment Response Index (MIRAI), to assess the ecological condition of the macroinvertebrate assemblage. The corresponding null-hypothesis would be that the different habitat requirements of the macroinvertebrate taxa in terms of velocity and substratum type cannot be used to refine the macroinvertebrate taxa’s preference ratings in the MIRAI, to assess the ecological condition of the macroinvertebrate assemblage. The preference ratings, based on the HSCs as well as information from the literature and personal experience, determined in Chapter 3 were successfully used in MIRAI v2 to determine the ecological condition of the macroinvertebrate assemblage at 44 sites spanning a range of conditions. The high correlation values (>0.9) for the different MIRAI metrics tested clearly indicates that the null hypothesis can be rejected.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS.

The aim of this project was to determine the habitat requirements of selected macroinvertebrate families, by determining the preferred ranges of water depth, velocity and temperature as well at the substratum types required by these taxa. These results were then used to update the MIRAI. It is also foreseen that these results will assist in the setting of the flow component of the Ecological Reserve.

A review of the findings of the thesis is presented in this chapter. Final conclusions are drawn to the research questions presented at the beginning of the thesis. This study highlighted the scarcity as well as the need for environmental requirements and life history information for the South African freshwater macroinvertebrates. This is of particular importance in the determination of the Ecological Reserve where the confidence in the Ecological Reserve requirements are only as good as the data used to derive the flow regime. Although the environmental requirement information is essential in both (DRIFT and HFSR) methods (O’Keeffe *et al*. 2002, King *et al*. 2004) used to determine the Ecological Reserve, the life history information is crucial in the DRIFT method (King *et a*l. 2004). In addition to the velocity, substratum, depth and geographical distribution, the DRIFT method also require information on seasonality, breeding cycle and habits (e.g. when and where the eggs are laid, timing of emergence etc.). The method also requires the substantiation of the perceived requirements (King *et al*. 2004).

5.1 CHAPTER 2: The geographical distribution of Ephemeroptera, Trichoptera, Coleoptera and Diptera in South Africa

Although by no means conclusive, a good indication of the distribution ranges of the families in the four orders under investigation was obtained. The need to archive voucher specimens, not only for new or unidentified taxa, but also to validate the range distributions of known taxa is highlighted. An example of this is the range extension in a number of taxa such as the Calamoceratidae (Trichoptera) and the Ptilodactylidae (Coleoptera) as well as the identification of questionable distribution records for a number of mostly south-western Cape endemic caddisflies such as Barbarochthonidae, Hydrosalpingidae, Sericostomatidae and Glossosomatidae. These questionable records can be investigated and corrected only if there are preserved specimens available. It seems as if there are certain families such as the Oligoneuriidae, Teloganodidae, and particularly, a number of the cased caddisflies that are more prone to being misidentified than others. It is important that the SASS training courses offered in South Africa highlight these problems so that samplers are aware of the pitfalls. Another important aspect is to make samplers aware of the importance of lodging voucher specimens and to preserve unfamiliar taxa for proper identification by experts.

The scarcity in distribution records for a number of families, most notably that of the Hydrosalpingidae (Trichoptera), Ptilodactylidae, Limnichidae (Coleoptera) and Ephemeridae (Ephemeroptera) was highlighted. There are a number of families with disjunct distributions such as: Prosopistomatidae (Ephemeroptera), Limnichidae (Coleoptera) and Blephariceridae (Diptera). Although the distribution of the Blephariceridae can be explained by their rather stringent habitat requirements (Barraclough and Londt 1985, Harrison 2003, Harrison *et al.* 2003a, Harrison *et al*. 2003c, Picker *et al*. 2003) the distribution of the Prosopistomatidae and the Limnichidae cannot be explained readily and might be more a function of insufficient collection rather than strict habitat requirements.

The hypothesis tested in this chapter was that the macroinvertebrate assemblage structure can be differentiated based on Ecoregion delineation and geomorphological zonation. The results indicated that this is true for certain taxa while other taxa have a countrywide distribution and have been recorded from most geomorphological zones. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on Ecoregion and geomorphological zone. The null hypothesis that the macroinvertebrate assemblage structure cannot be differentiated on Ecoregion delineation and geomorphological zonation is therefore rejected.

5.2 CHAPTER 3: Environmental requirements of selected Ephemeroptera, Trichoptera, Coleoptera and Diptera in South Africa

The results from this chapter indicated that environmental factors such as velocity, pH, temperature, latitude and longitude, as well as Ecoregion, geomorphological zone and substratum type were determining factors in the distribution patterns of the insect families under consideration. Not all of the factors were important for all of the families. While certain common families such as the baetid mayflies and the chironomid midges showed no preference for any of the environmental factors under consideration, others such as the blackflies (Simuliidae) and net winged midges (Blephariceridae) are associated with very fast flowing water over cobbles, the squaregill mayflies (Caenidae) with the GSM biotope and the predacious diving beetles (Dytiscidae) with vegetation. The distribution of taxa with a more limited geographical range such as the more subtropical Calamoceratidae and the burrowing mayflies (Ephemeridae and Polymitarcyidae) are associated with Ecoregion as well as latitude and longitude while the distribution of the southwestern Cape endemic mayflies (Teloganodidae) and cased caddisflies (Sericostomatidae, Glossosomatidae) are also associated with low pH values. The importance of noting the developmental stage of insects such as larva, pupa and adult is highlighted most notably by the different environmental requirements of the beetles where the larval and adult stages sometimes have different requirements.

The hypothesis tested in this chapter was that the macroinvertebrate assemblage structure can be differentiated based on environmental factors such as substratum, depth, and velocity as well as physico-chemical parameters. The results indicated that this is true for certain taxa but not for others. It is also clear that certain environmental factors play a role in the distribution of certain taxa but not others (e.g. temperature is a determining factor for Blephariceridae, but not for Simuliidae). Interestingly enough depth was not significant factor in determining the distribution of the insects under consideration. However, the macroinvertebrate assemblage structure as a whole can be differentiated based on a combination of environmental factors and the null hypothesis that the macroinvertebrate assemblage structure cannot be differentiated based in environmental factors is therefore rejected.

The information obtained in this chapter provides a first step in setting habitat requirements for selected families of Ephemeroptera, Trichoptera, Coleoptera and Diptera. There is a need for more data on certain families such as Prosopistomatidae, Sericostomatidae, Glossosomatidae, Haliplidae, Ephydridae, Syrphidae and others not included in this chapter. The only way of obtaining data for these families is to actively target localities where they are known to occur. The limitations of the sampling protocol that actively excluded areas impacted by pollution, was highlighted by the absence of taxa such as Syrphidae that are known to occur in organically polluted water (Harrison *et al*. 2003b).

Although depth does not seem to be a determining factor in the occurrence of the macroinvertebrate families investigated here, there is still a need for investigating the effect of particularly shallower depths as there might be a threshold value below which the macroinvertebrates could potentially be affected. Only five samples were taken at very shallow (<10 cm) locations, and no samples at depths <5 cm, potentially missing the threshold depth. It is very important to actually determine such a threshold value in order to assess the impact of flow reductions during the Ecological Water Requirements (EWR) process of Ecological Reserve Determinations. There is a real danger of damaging the riverine macroinvertebrate communities if depth is ignored and the focus is solely placed on substratum and velocity as there can still be water of a suitable velocity but the depth might be too shallow to enable long-term survival of the resident macroinvertebrates.

5.3 CHAPTER 4: Update of the Macroinvertebrate Response Assessment Index (MIRAI).

Although certain families such as Baetidae, Leptoceridae and Chironomidae for instance do not show strong preference for some of the metric groups, they are likely to contain species that exhibit highly specialised environmental requirements (Matthew 1968, Skoroszewski and de Moor 1999, Suter *et al*. 2002, de Moor and Scott 2003, Bouchard 2004, Morse 2004, Horta *et al*. 2009). In these instances it will be essential to use genus or species level identifications and redo the HSCs for these species. Recent work by Barber-James and Pereira-da-Conceicoa (2016) on Teloganodidae highlights the importance of species level identifications. Another important factor is that different life stages of the invertebrate taxa do not necessarily have the same environmental requirements. As an example the adult whirligig beetles (Gyrinidae) are mostly found on the water surface whereas the larvae mostly occur in the vegetation (Biström 1985, Picker *et al*. 2003, Stals 2008b). The same might be true for other Coleoptera and Diptera as well. Although the River EcoStatus Monitoring Programme (REMP) uses SASS5 as the monitoring tool for aquatic macroinvertebrates, more detailed information is required when doing Environmental Impact Assessments and Ecological Reserve Determinations (de Moor 2002, Barber-James and Pereira-da-Conceicoa 2015). The samples collected during this study are still available and it is recommended that they be identified to genus or species level where possible and that HSCs and preference ratings be determined on genus or species level. This information can then be used in the Fish Invertebrate Flow Habitat Assessment model (FIFHA) (Kleynhans and Thirion 2015c) during the Ecological Reserve Determination process.

Ever since the MIRAI was originally developed, it was recognised that the preference ratings for velocity and substratum types needed to be updated based on real data, rather than just a combination of very limited information from the literature and personal experience. Although this study only partly succeeded in gathering field-based data on the habitat requirements of the riverine macroinvertebrate taxa it has been a first step in improving the preference ratings used in the MIRAI. This study should be broadened to include taxa not yet studied, and redesigned to include the water surface and water column as a “substratum” type for macroinvertebrates. This will enable one to distinguish between the use of the water surface, in particular, and the marginal vegetation as a habitat for certain invertebrate taxa.

The following hypothesis was tested: The different habitat requirements of the invertebrate taxa in terms of velocity and substratum type can be used to refine the macroinvertebrate taxa’s preference ratings in the Macroinvertebrate Assessment Response Index, to assess the ecological condition of the macroinvertebrate assemblage. The corresponding null-hypothesis would be that the different habitat requirements of the invertebrate taxa in terms of velocity and substratum type cannot be used to refine the macroinvertebrate taxa’s preference ratings in the MIRAI, to assess the ecological condition of the macroinvertebrate assemblage. The preference ratings, based on the HSCs as well as information from the literature and personal experience (determined in Chapter 3), were successfully used in MIRAI v2 to determine the ecological condition of the macroinvertebrate assemblage at 44 sites spanning a range of conditions. The high correlation values (>0.9) for the different MIRAI metrics tested clearly indicates that the null hypothesis can be rejected.

5.4 Recommendations and Future Research.

The geographical distribution presented in Chapter 2 is based on information available in January 2014. More information has become available since then. Although the Rivers Database is currently non-functional, the information added until October 2015 is available on the Resource Quality Information Services (RQIS) website (https://www.dwa.gov.za/iwqs/rhp/database.html). This information as well as SASS5 data sheets submitted but not captured should be used to update the information used to map the distribution ranges. The distribution ranges can then be updated and the KML files as well as distribution maps placed on the RQIS website.

The gaps identified in this thesis should be filled by actively targeting areas with limited or no data such as the Namaqua Highlands and Northern Plateau. A concerted effort should be made to collect information on the distribution of taxa with limited records by actively sampling habitats where they are likely to occur.

A sampling programme should be designed and implemented to include the water surface as a possible habitat. The study area should also be expanded to include polluted sites in order to collect data on taxa such as Syrphidae (Harrison *et al*. 2003a) that are indicative of pollution. Information from such a sampling programme can be used to develop HSCs and preference ratings for these taxa. Existing samples should be identified to genus or species level and HSCs and preference values determined for these genera and species where possible. This information can then be used to improve the MIRAI and also feed into the FIFHA (Kleynhans and Thirion 2015c) for use during EWR determinations. More samples should be collected for “rarer” taxa and the HSCs and preference values refined if required. A wider range of depths should be included in order to determine a possible “threshold” depth. Information collected can then be used to feed into the RHAMM (Kleynhans and Thirion 2015b) and FIFHA (Kleynhans and Thirion 2015c) models.

The first step in the Ecological Classification process is the determination of reference conditions for each of the components (Kleynhans and Louw 2007). The use of realistic reference conditions is crucial in the application of the MIRAI (Thirion 2007). Sets of reference sites have traditionally been used to create a benchmark against which the current condition of a river can be measured (Rosenberg *et al*. 1999, Chessman and Royal 2004, Dallas 2004b, Thirion 2007). The problem with this approach is that it is extremely difficult to identify possible reference sites due to widespread human modification of river systems (Chessman and Royal 2004, Chessman 2006, Chessman *et al*. 2008). Locally, the only likely minimally impacted sites are mostly located in the upper reaches of the rivers and cannot be used as a reference for sites further downstream. The problem is even larger in the Highveld region of South Africa where the river sources are in or near industrial, urban and mining areas. There is a very real danger of making comparisons against a reference condition that already exhibits sometimes substantial and poorly defined impacts resulting in an erroneously high Ecological Category (Chessman and Royal 2004). The problem with reference sites has been overcome to a certain degree by the development of reference conditions through a variety of methods such as the use of historical data, professional judgement and more recently the concept of environmental filters (Chessman and Royal 2004, Chessman 2006, Chessman *et al*. 2008). There is very limited information on the distribution of macroinvertebrates pre 1950, and even less from the 1930s and earlier. This implies that it is not practical to rely solely on historical information to determine reference conditions for South African riverine macroinvertebrates. One way of actually assisting in the process of determining reference conditions for South African macroinvertebrates is to use the distribution ranges presented in Chapter 2 to derive a list of possible reference taxa per Level II Ecoregion, geomorphological zone and altitude range to choose from for a particular site. These lists should be included in MIRAI v2 as well as the RHAMM model. It would then be up to the user to compile the likely reference condition for a site, based not only on this list but also the natural characteristics of the site or reach in question. Ideally these reference conditions should be placed in a central location such as the DWS website, where other researchers can access it.

The effects of the changes in the flow modification and habitat modification metric group results between the 2 MIRAI versions should be investigated. The following questions should be answered:

- Does it have an impact on the explanation for the Ecological Condition or impacts at the site?
- Does it explain the impacts more realistically than the information obtained using MIRAI v1?

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ANNEXURE

Table A.1: The occurrence of Freshwater Macroinvertebrate taxa per geomorphological zone and altitude (m a.m.s.l).

Taxon	**Source**	**High-Gradient Mountain**	**Mountain**	**Transitional**	**Upper Foothills**	**Lower Foothills**	**Lowland**	**MIN ALT**	**MAX ALT**
Porifera			🟨	🟨	🟨	🟨	🟨	20	1700
Coelenterata									
Turbellaria		🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Oligochaeta		🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Hirudinea		🟨	🟨	🟨	🟨	🟨	🟨	0	1900
Amphipoda	🟨	🟨	🟨	🟨	🟨	🟨	🟨	10	3100
Potamonautidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2600
Atyidae			🟨	🟨	🟨	🟨	🟨	0	1600
Paleomonidae					🟨	🟨	🟨	0	1500
Hydracarina			🟨	🟨	🟨	🟨	🟨	10	2200
Notonemouridae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Perlidae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Baetidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Caenidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Ephemeridae					🟨	🟨	🟨	80	1500
Heptageniidae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Leptophlebiidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Oligoneuridae		🟨	🟨	🟨	🟨	🟨	🟨	0	3132
Polymitarcyidae			🟨	🟨	🟨	🟨	🟨	20	1800
Prosopistomatidae		🟨	🟨	🟨	🟨	🟨	🟨	10	2200
Telagonodidae		🟨	🟨	🟨	🟨	🟨	🟨	0	1100
Trichorythidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Calopterygidae				🟨	🟨	🟨	🟨	20	2200
Chlorocyphidae		🟨	🟨	🟨	🟨	🟨	🟨	20	1700
Chlorolestidae			🟨	🟨	🟨	🟨	🟨	0	2700
Coenagrionidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Lestidae			🟨	🟨	🟨	🟨	🟨	20	1600
Platycnemidae			🟨	🟨	🟨	🟨	🟨	0	3200
Protoneuridae				🟨	🟨	🟨	🟨	0	2100
Aeshnidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Corduliidae			🟨	🟨	🟨	🟨	🟨	20	1700
Gomphidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Libellulidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2500
Pyralidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Belostomatidae			🟨	🟨	🟨	🟨	🟨	0	1900
Corixidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Gerridae		🟨	🟨	🟨	🟨	🟨	🟨	0	2600
Hydrometridae				🟨	🟨	🟨	🟨	0	1800
Naucoridae		🟨	🟨	🟨	🟨	🟨	🟨	0	2700
Nepidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2200
Notonectidae			🟨	🟨	🟨	🟨	🟨	0	2200
Pleidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2500
Veliidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Corydalidae		🟨	🟨	🟨	🟨	🟨		20	2000
Sialidae				🟨	🟨	🟨		10	1700

Table A.1 (continued): The occurrence of Freshwater Macroinvertebrate taxa per geomorphological zone and altitude (m a.m.s.l).

Taxon	Source	High-Gradient Mountain	Mountain	Transitional	Upper Foothills	Lower Foothills	Lowland	MIN ALT	MAX ALT
Dipseudopsidae			🟨	🟨	🟨	🟨	🟨	0	1700
Ecnomidae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Hydropsychidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Philopotamidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2200
Polycentropodidae		🟨	🟨	🟨	🟨	🟨	🟨	20	1800
Psychomyiidae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Barbarochthonidae		🟨	🟨	🟨	🟨	🟨		10	1000
Calamoceratidae				🟨	🟨	🟨	🟨	0	1300
Glossosomatidae		🟨	🟨	🟨	🟨	🟨		0	900
Hydroptilidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Hydropsalpingidae				🟨	🟨			250	1400
Lepidostomatidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3000
Leptoceridae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Petrothrincidae		🟨	🟨	🟨	🟨	🟨		30	900
Pisuliidae		🟨	🟨	🟨	🟨	🟨	🟨	20	2000
Sericostomatidae		🟨	🟨	🟨	🟨	🟨	🟨	20	1900
Dytiscidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Elmidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3500
Gyrinidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Haliplidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2000
Helodidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Hydraenidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Hydrophilidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2600
Limnichidae		🟨	🟨	🟨	🟨	🟨	🟨	140	1700
Psephenidae		🟨	🟨	🟨	🟨	🟨	🟨	10	1800
Athericidae		🟨	🟨	🟨	🟨	🟨	🟨	10	3200
Blepharoceridae		🟨	🟨	🟨	🟨	🟨	🟨	20	3200
Ceratopogonidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Chironomidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Culicidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Dixidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Empididae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Ephydridae		🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Muscidae		🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Psychodidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3100
Simuliidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Syrphidae				🟨	🟨	🟨	🟨	0	1700
Tabanidae		🟨	🟨	🟨	🟨	🟨	🟨	0	2900
Tipulidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Ancylidae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	2300
Bulilinae	🟨			🟨	🟨	🟨	🟨	0	2700
Hydrobiidae				🟨	🟨	🟨	🟨	0	300
Lymnaeidae	🟨			🟨	🟨	🟨	🟨	0	1700
Physidae				🟨	🟨	🟨	🟨	0	2900
Planorbinae	🟨	🟨	🟨	🟨	🟨	🟨	🟨	0	3200
Thiaridae				🟨	🟨	🟨	🟨	10	1600
Viviparidae							🟨	0	50
Corbiculidae			🟨	🟨	🟨	🟨	🟨	0	1800
Sphaeridae			🟨	🟨	🟨	🟨	🟨	0	1900
Unionidae					🟨	🟨	🟨	0	1800

ANNEXURE

Table A.2: The occurrence of Freshwater Macroinvertebrate taxa per level II Ecoregion.

Taxon	1.01	1.02	1.03	1.04	2.01	2.02	2.03	2.04	3.01	3.02	3.03	3.04	3.05	3.06	3.07	3.08	3.09	3.10	4.01	4.02	4.03	4.04	4.05	4.06	5.01	5.02	6.01	6.02	7.01	**7.02**	**7.03**	**7.04**	**7.05**	**7.06**
Porifera														🟨	🟨							🟨			🟨				🟨			🟨	🟨	🟨
Coelenterata																																		
Turbellaria	🟨					🟨		🟨	🟨	🟨	🟨			🟨	🟨					🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨	🟨	🟨	🟨
Oligochaeta	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Hirudinea	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨					🟨	🟨		🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Amphipoda									🟨													🟨					🟨					🟨		
Potamonautidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨		🟨	🟨		🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Atyidae	🟨		🟨	🟨		🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨		🟨		🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Paleomonidae	🟨					🟨		🟨	🟨		🟨			🟨				🟨																
Hydracarina	🟨	🟨	🟨			🟨		🟨	🟨		🟨			🟨	🟨	🟨	🟨	🟨		🟨		🟨		🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Notonemouridae																						🟨												
Perlidae						🟨		🟨	🟨	🟨	🟨			🟨	🟨					🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Baetidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Caenidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Ephemeridae																																		
Heptageniidae	🟨	🟨				🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨	🟨		🟨		🟨	🟨	🟨			🟨	🟨		🟨	🟨	🟨	🟨	🟨
Leptophlebiidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Oligoneuridae	🟨	🟨				🟨		🟨	🟨		🟨			🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨				🟨	🟨		🟨		🟨		🟨
Polymitarcyidae				🟨				🟨	🟨		🟨			🟨	🟨		🟨					🟨	🟨				🟨					🟨		
Prosopistomatidae														🟨	🟨		🟨					🟨		🟨										
Telagonodidae																																		
Trichorythidae	🟨					🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨	🟨		🟨		🟨	🟨	🟨				🟨	🟨	🟨	🟨	🟨	🟨	🟨
Calopterygidae						🟨		🟨	🟨						🟨	🟨	🟨					🟨					🟧							
Chlorocyphidae	🟨		🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨			🟨		🟨	🟨				🟨	🟨	🟨		🟨	🟨	🟨	🟨
Chlorolestidae						🟨		🟨	🟨		🟨				🟨					🟨		🟨	🟨						🟨			🟨	🟨	🟨
Coenagrionidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Lestidae	🟨	🟨	🟨					🟨	🟨		🟨				🟨					🟨		🟨					🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Platycnemidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨							🟨	🟨				🟨					🟨		
Protoneuridae						🟨		🟨			🟨				🟨							🟨										🟨	🟨	🟨
Aeshnidae	🟨	🟨	🟨			🟨		🟨	🟨		🟨			🟨	🟨					🟨		🟨	🟨	🟨			🟨	🟨	🟨		🟨	🟨	🟨	🟨
Corduliidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨			🟨		🟨	🟨	🟨	🟨		🟨		🟨	🟨			🟨	🟨	🟨	🟨
Gomphidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Libellulidae	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨		🟨	🟨	🟨	🟨
Pyralidae						🟨		🟨			🟨			🟨	🟨		🟨			🟨		🟨	🟨	🟨			🟨					🟨		🟨
Belostomatidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨				🟨	🟨	🟨		🟨	🟨	🟨	🟨
Corixidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨		🟨			🟨	🟨		🟨	🟨				🟨	🟨	🟨	🟨		🟨	🟨	🟨		🟨	🟨	🟨	🟨
Gerridae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨		🟨	🟨		🟨		🟨	🟨	🟨		🟨	🟨	🟨	🟨
Hydrometridae	🟨		🟨					🟨	🟨		🟨				🟨	🟨						🟨		🟨	🟨		🟨	🟨			🟨	🟨	🟨	
Naucoridae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨				🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Nepidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨		🟨				🟨	🟨				🟨	🟨	🟨			🟨	🟨	🟨
Notonectidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨				🟨	🟨	🟨		🟨	🟨	🟨	🟨
Pleidae		🟨						🟨	🟨	🟨	🟨			🟨	🟨							🟨	🟨	🟨			🟨		🟨			🟨	🟨	🟨
Veliidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Corydalidae																																		
Sialidae																																		
Dipseudopsidae									🟨						🟨	🟨				🟨		🟨										🟨		
Ecnomidae	🟨					🟨		🟨			🟨			🟨	🟨	🟨						🟨		🟨	🟨			🟨	🟨		🟨	🟨	🟨	🟨
Hydropsychidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Philopotamidae	🟨	🟨	🟨			🟨		🟨	🟨		🟨			🟨	🟨					🟨		🟨	🟨	🟨			🟨	🟨	🟨		🟨	🟨	🟨	🟨
Polycentropodidae								🟨		🟨	🟨				🟨			🟨		🟨		🟨							🟨			🟨	🟨	🟨
Psychomyiidae		🟨									🟨			🟨	🟨					🟨		🟨					🟨				🟨			
Barbarochthonidae																																		
Calamoceratidae								🟨	🟨						🟨							🟨	🟨											
Glossosomatidae																																		
Hydroptilidae	🟨					🟨		🟨	🟨		🟨			🟨	🟨		🟨			🟨		🟨	🟨	🟨			🟨	🟨				🟨	🟨	🟨
Hydropsalpingidae																																		
Lepidostomatidae						🟨		🟨	🟨		🟨				🟨					🟨		🟨	🟨				🟨				🟨	🟨	🟨	
Leptoceridae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Petrothrincidae																																		
Pisuliidae																						🟨										🟨		
Sericostomatidae																																		
Dytiscidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Elmidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨		🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨
Gyrinidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Haliplidae	🟨					🟨		🟨			🟨			🟨	🟨					🟨		🟨	🟨	🟨				🟨	🟨		🟨	🟨	🟨	🟨
Helodidae	🟨							🟨	🟨	🟨	🟨			🟨	🟨	🟨				🟨		🟨	🟨		🟨				🟨		🟨	🟨	🟨	🟨
Hydraenidae	🟨			🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨					🟨	🟨	🟨			🟨		🟨		🟨	🟨	🟨	🟨
Hydrophilidae	🟨	🟨	🟨	🟨		🟨		🟨	🟨		🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨		🟨	🟨	🟨	🟨
Limnichidae															🟨							🟨	🟨									🟨		🟨
Psephenidae						🟨		🟨	🟨		🟨				🟨					🟨		🟨	🟨	🟨				🟨		🟨	🟨	🟨	🟨	🟨
Athericidae	🟨					🟨		🟨	🟨		🟨			🟨	🟨		🟨			🟨		🟨	🟨	🟨			🟨	🟨		🟨	🟨	🟨	🟨	🟨
Blepharoceridae																				🟨		🟨										🟨		
Ceratopogonidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨		🟨	🟨	🟨	🟨
Chironomidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Culicidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨		🟨	🟨	🟨	🟨		🟨	🟨	🟨		🟨	🟨	🟨	🟨
Dixidae			🟨					🟨	🟨						🟨							🟨	🟨				🟨					🟨	🟨	🟨
Empididae								🟨			🟨				🟨		🟨					🟨		🟨			🟨							🟨
Ephydridae											🟨				🟨	🟨																🟨	🟨	🟨
Muscidae	🟨	🟨				🟨			🟨		🟨			🟨	🟨						🟨	🟨	🟨					🟨	🟨	🟨		🟨	🟨	🟨
Psychodidae	🟨	🟨				🟨			🟨		🟨			🟨	🟨	🟨	🟨					🟨			🟨								🟨	🟨
Simuliidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Syrphidae			🟨						🟨		🟨				🟨												🟨					🟨	🟨	
Tabanidae	🟨		🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Tipulidae		🟨						🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨			🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨		🟨	🟨	🟨
Ancylidae	🟨	🟨				🟨		🟨	🟨	🟨	🟨				🟨		🟨	🟨				🟨	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Bulilinae		🟨							🟨		🟨			🟨	🟨	🟨	🟨					🟨		🟨			🟨	🟨	🟨		🟨	🟨	🟨	🟨
Hydrobiidae																																		
Lymnaeidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨				🟨					🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Physidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨		🟨			🟨		🟨			🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨
Planorbinae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨			🟨		🟨	🟨	🟨			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Thiaridae	🟨	🟨				🟨		🟨	🟨	🟨	🟨			🟨	🟨	🟨	🟨					🟨	🟨				🟨		🟨			🟨	🟨	🟨
Viviparidae																																		
Corbiculidae	🟨	🟨	🟨			🟨		🟨	🟨	🟨	🟨				🟨	🟨	🟨			🟨							🟨	🟨	🟨	🟨		🟨	🟨	🟨
Sphaeridae	🟨	🟨				🟨		🟨	🟨		🟨			🟨	🟨		🟨				🟨	🟨	🟨					🟨	🟨		🟨	🟨	🟨	🟨
Unionidae		🟨									🟨				🟨													🟨						

Table A.2 (Continued): The occurrence of Freshwater Macroinvertebrate taxa per level II Ecoregion.

Taxon	**8.01**	**8.02**	**8.03**	**8.04**	**8.05**	**8.06**	**9.01**	**9.02**	**9.03**	**9.04**	**9.05**	**9.06**	**10.01**	**10.02**	**10.03**	**11.01**	**11.02**	**11.03**	**11.04**	**11.05**	**11.06**	**11.07**	11.08	11.09	11.10	12.01	13.01	13.02	13.03
Porifera	■		■					■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■			
Coelenterata																													
Turbellaria	■			■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■			■
Oligochaeta	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Hirudinea	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■		■	■
Amphipoda													■	■												■	■	■	■
Potamonautidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		■	■
Atyidae			■	■	■	■		■	■			■	■		■	■	■	■	■	■			■	■	■	■		■	■
Paleomonidae																										■			
Hydracarina	■		■		■	■		■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■		■	■
Notonemouridae								■		■			■	■															
Perlidae								■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		■			
Baetidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Caenidae			■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ephemeridae												■														■			
Heptageniidae	■		■			■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	
Leptophlebiidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Oligoneuridae								■	■			■	■	■	■		■				■					■			
Polymitarcyidae			■	■					■			■	■	■	■	■	■	■		■				■		■	■	■	
Prosopistomatidae								■	■	■	■	■	■	■	■	■	■	■	■	■						■			
Telagonodidae																													
Trichorythidae			■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Calopterygidae								■	■	■	■															■		■	
Chlorocyphidae			■		■	■		■	■	■	■	■	■	■	■		■	■	■	■			■	■		■			
Chlorolestidae					■	■		■			■	■	■	■	■	■	■	■		■	■		■	■		■			
Coenagrionidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Lestidae					■			■		■	■	■	■		■	■	■	■		■				■	■				
Platycnemidae			■		■	■		■					■	■	■	■								■					
Protoneuridae								■	■	■		■	■	■	■		■	■					■			■			
Aeshnidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■
Corduliidae	■		■		■			■	■	■		■	■	■	■	■	■	■	■	■			■		■	■		■	■
Gomphidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		■	
Libellulidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Pyralidae								■	■			■	■	■	■	■	■	■	■	■			■	■		■		■	
Belostomatidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■		■	■
Corixidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■
Gerridae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	
Hydrometridae					■	■			■		■			■		■	■	■		■			■	■	■				
Naucoridae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	
Nepidae			■	■	■	■		■	■		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		■	
Notonectidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Pleidae				■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	
Veliidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■
Corydalidae														■															
Sialidae																													
Dipseudopsidae								■								■	■	■		■				■		■		■	■
Ecnomidae			■	■	■			■	■		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		■	
Hydropsychidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Philopotamidae	■			■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■				■		■			
Polycentropodidae								■					■	■	■	■	■	■		■									
Psychomyiidae								■	■			■	■	■	■	■	■							■					
Barbarochthonidae																													
Calamoceratidae													■													■			■
Glossosomatidae																													
Hydroptilidae			■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		■			
Hydropsalpingidae																													
Lepidostomatidae								■					■	■		■								■					
Leptoceridae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■
Petrothrincidae																													
Pisuliidae					■								■	■		■								■					
Sericostomatidae																													
Dytiscidae	■		■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Elmidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		■			
Gyrinidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Haliplidae					■	■		■		■		■	■	■	■	■	■	■	■	■	■			■					■
Helodidae			■		■			■	■	■		■	■	■	■	■	■	■					■	■		■			
Hydraenidae	■				■	■		■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■			
Hydrophilidae	■		■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Limnichidae													■																
Psephenidae	■		■		■	■		■	■	■	■	■	■	■	■		■	■	■	■	■			■					
Athericidae								■	■	■	■	■	■	■	■	■	■	■						■		■			
Blepharoceridae													■	■															
Ceratopogonidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Chironomidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Culicidae	■				■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■
Dixidae								■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■			
Empididae								■	■				■	■		■	■	■		■					■				
Ephydridae					■								■			■	■	■		■						■	■		
Muscidae			■	■	■			■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Psychodidae								■					■	■	■	■	■	■					■			■			
Simuliidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Syrphidae					■											■		■							■				■
Tabanidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■					■	■	■	■		■	■
Tipulidae			■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	
Ancylidae					■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■			
Bulilinae	■								■			■			■	■	■	■		■	■		■		■	■	■	■	■
Hydrobiidae																													
Lymnaeidae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■		■	■
Physidae	■		■	■	■	■		■	■	■	■	■		■	■	■		■		■	■	■	■	■	■	■			■
Planorbinae	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■
Thiaridae	■		■	■	■			■	■	■		■				■		■		■			■		■	■		■	■
Viviparidae																												■	
Corbiculidae			■	■	■	■			■			■			■	■	■	■		■			■	■	■	■		■	
Sphaeridae			■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		■	■
Unionidae			■									■				■	■	■		■					■	■			

Table A.2 (Continued): The occurrence of Freshwater Macroinvertebrate taxa per level II Ecoregion.

Taxon	14.01	14.02	14.03	14.04	14.05	14.06	14.07	14.08	15.01	15.02	15.03	15.04	15.05	15.06	15.07	16.01	16.02	**16.03**	**16.04**	**16.05**	**16.06**	**16.07**	**16.08**	**17.01**	**17.02**	**17.03**	**17.04**	**18.01**	**18.02**	**18.03**	**18.04**
Porifera							■					■				■		■			■			■							
Coelenterata																															
Turbellaria		■	■		■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		
Oligochaeta	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	
Hirudinea		■	■			■	■		■		■	■	■			■	■	■	■		■	■		■	■	■			■		
Amphipoda					■		■					■		■	■	■		■	■	■	■			■	■	■		■			
Potamonautidae	■	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Atyidae		■	■		■	■	■				■				■	■	■	■	■	■	■	■		■		■	■				
Paleomonidae					■	■										■		■	■					■	■	■	■				
Hydracarina	■	■	■	■	■	■	■		■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■			■		
Notonemouridae		■								■	■		■	■	■	■	■		■	■		■				■					
Perlidae	■	■	■		■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Baetidae	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Caenidae	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	
Ephemeridae							■					■				■		■	■							■					
Heptageniidae		■	■	■	■	■	■			■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Leptophlebiidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Oligoneuridae		■				■	■					■		■	■	■	■	■	■	■	■	■		■	■	■					
Polymitarcyidae		■				■	■		■						■	■	■	■	■	■				■		■					
Prosopistomatidae		■				■	■					■			■	■	■	■	■	■	■		■	■		■					
Telagonodidae																					■	■						■	■		
Trichorythidae	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		
Calopterygidae						■										■	■	■		■	■			■	■						
Chlorocyphidae		■	■		■	■	■					■			■	■	■	■	■	■	■	■	■	■	■	■			■		
Chlorolestidae						■	■				■	■	■	■	■	■		■	■	■	■	■	■	■	■	■					
Coenagrionidae		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Lestidae		■	■				■		■			■	■		■	■	■	■	■		■	■		■	■	■					
Platycnemidae					■	■	■	■			■			■	■	■	■	■	■		■	■		■		■					
Protoneuridae		■					■										■	■				■		■	■	■			■		
Aeshnidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Corduliidae						■	■			■		■				■	■	■	■		■	■	■	■	■	■			■		
Gomphidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		
Libellulidae	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■			■		
Pyralidae		■	■	■	■	■	■					■	■	■	■	■	■	■	■		■	■		■	■	■			■		
Belostomatidae		■	■	■	■	■	■		■		■	■			■	■	■	■	■		■	■	■	■	■	■			■		
Corixidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Gerridae		■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Hydrometridae					■		■		■		■	■			■	■	■	■	■	■		■	■	■	■	■			■		
Naucoridae		■				■	■		■	■	■	■	■		■	■	■	■	■	■	■	■	■	■		■			■	■	
Nepidae		■	■		■	■	■				■	■	■	■	■	■	■	■	■	■	■	■		■	■	■					
Notonectidae		■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■		■	■	■	
Pleidae		■				■	■		■	■	■	■	■	■	■	■	■	■	■		■			■	■	■			■	■	
Veliidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	
Corydalidae		■									■	■						■	■			■				■					
Sialidae																■		■	■												
Dipseudopsidae					■							■			■	■		■						■							
Ecnomidae		■			■	■	■				■	■	■	■	■	■	■	■	■		■			■		■		■	■		
Hydropsychidae	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Philopotamidae		■			■	■	■					■	■		■	■	■	■	■	■	■	■		■	■	■			■		
Polycentropodidae							■					■			■	■	■	■	■	■	■			■		■					
Psychomyiidae										■	■	■		■	■	■						■	■	■					■		
Barbarochthonidae																															
Calamoceratidae																		■													
Glossosomatidae																															
Hydroptilidae		■	■	■		■	■		■			■		■	■	■	■	■	■	■	■	■		■	■	■		■	■		
Hydropsalpingidae																															
Lepidostomatidae														■	■	■	■	■	■		■	■		■							
Leptoceridae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Petrothrincidae																															
Pisuliidae					■	■					■				■	■		■			■	■		■							
Sericostomatidae							■							■	■	■	■		■		■			■							
Dytiscidae	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Elmidae		■	■	■	■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		
Gyrinidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Haliplidae		■				■			■			■			■	■	■	■	■	■				■	■	■			■	■	
Helodidae		■				■	■	■	■		■	■		■	■	■		■	■	■	■	■		■					■		
Hydraenidae	■	■				■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	
Hydrophilidae	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Limnichidae																															
Psephenidae	■	■				■	■					■			■	■	■	■	■	■	■	■		■		■			■		
Athericidae		■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■			■	■	
Blepharoceridae						■	■				■			■	■	■	■	■	■	■	■	■		■							
Ceratopogonidae		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Chironomidae	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Culicidae		■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Dixidae	■	■				■	■		■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■					
Empididae		■					■		■		■	■		■	■	■	■	■	■					■		■		■	■		
Ephydridae		■				■			■					■	■	■	■	■	■					■	■	■			■	■	
Muscidae		■				■	■		■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Psychodidae		■				■	■				■	■		■	■	■	■	■	■		■	■		■	■	■					
Simuliidae		■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
Syrphidae		■				■	■									■	■	■						■	■						
Tabanidae		■	■	■	■	■	■		■		■	■		■	■	■	■	■	■	■	■	■		■	■	■			■	■	
Tipulidae		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■		
Ancylidae		■	■		■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	
Bulilinae		■			■	■	■		■	■					■	■	■	■	■				■	■	■	■			■	■	
Hydrobiidae																									■	■					
Lymnaeidae		■	■	■	■	■	■		■			■			■	■		■	■		■			■	■	■			■	■	
Physidae		■	■		■	■	■		■		■	■		■		■	■	■	■		■	■		■	■	■			■		
Planorbinae		■	■	■	■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	
Thiaridae						■	■									■		■	■					■	■	■					
Viviparidae																															
Corbiculidae		■				■	■				■	■	■			■	■	■	■		■	■		■		■			■		
Sphaeridae		■			■	■	■		■		■	■	■		■	■	■	■	■		■	■		■	■	■			■	■	
Unionidae		■					■					■	■				■	■			■	■				■		■	■	■	

Table A.2 (Continued): The occurrence of Freshwater Macroinvertebrate taxa per level II Ecoregion.

Taxon	**19.01**	**19.02**	**19.03**	**19.04**	**19.05**	**19.06**	**19.07**	**19.08**	**19.09**	**19.10**	**20.01**	20.02	21.01	21.02	21.03	21.04	21.05	22.01	22.02	22.03	22.04	22.05	23.01	23.02	23.03	23.04
Porifera		●									●															
Coelenterata																										
Turbellaria	●	●	●	●	●	●	●	●	●	●	●	●		●				●	●		●		●	●	●	●
Oligochaeta	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●	●	●	●	●	●	●	●
Hirudinea	●		●	●	●	●	●	●	●		●	●							●		●		●	●	●	●
Amphipoda	●	●	●	●	●	●					●	●		●					●	●	●		●	●	●	
Potamonautidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Atyidae		●				●		●			●	●						●	●	●	●					
Paleomonidae						●					●	●						●	●				●		●	
Hydracarina	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●	●	●	●	●	●	●	●
Notonemouridae	●	●	●	●	●	●	●	●	●		●	●						●	●		●		●	●	●	●
Perlidae	●	●							●			●														
Baetidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Caenidae	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●
Ephemeridae																										
Heptageniidae	●	●	●	●		●			●			●							●		●		●	●	●	●
Leptophlebiidae	●	●	●	●	●	●	●	●	●			●		●				●	●		●		●	●	●	●
Oligoneuridae																										
Polymitarcyidae	●																									
Prosopistomatidae											●	●							●				●			
Telagonodidae	●	●	●	●		●	●	●	●			●		●				●	●		●		●	●	●	●
Trichorythidae	●	●	●	●		●			●			●		●					●		●		●	●	●	
Calopterygidae																										
Chlorocyphidae	●	●		●								●							●		●		●	●	●	
Chlorolestidae	●	●	●	●		●	●	●	●	●		●											●	●	●	
Coenagrionidae	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●
Lestidae	●	●							●		●	●	●						●				●		●	
Platycnemidae	●	●		●				●	●		●	●		●					●		●		●	●	●	
Protoneuridae	●								●		●	●							●				●	●		
Aeshnidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●			●		●		●	●	●	●
Corduliidae	●		●	●	●	●	●	●	●	●	●	●	●	●		●			●		●	●	●	●	●	●
Gomphidae	●	●	●	●	●	●	●	●	●	●		●		●	●	●		●	●		●	●	●	●	●	●
Libellulidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Pyralidae	●	●	●	●				●			●	●							●		●		●	●	●	
Belostomatidae	●	●	●	●		●	●		●		●	●		●					●	●	●		●	●	●	●
Corixidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Gerridae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●		●	●	●	●	●	●
Hydrometridae	●	●	●		●	●	●	●	●			●						●			●	●	●	●	●	
Naucoridae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●
Nepidae	●	●	●	●	●	●			●	●	●	●		●					●		●	●	●	●	●	●
Notonectidae	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●		●	●	●	●	●	●	●	●
Pleidae	●	●	●	●	●	●	●	●	●	●	●	●		●			●		●	●	●	●	●	●	●	●
Veliidae	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●		●	●	●	●	●	●	●	●	●
Corydalidae		●	●	●	●	●		●	●			●						●	●		●		●	●	●	
Sialidae				●								●							●					●	●	
Dipseudopsidae	●			●																			●			
Ecnomidae	●	●		●		●	●	●	●		●	●		●	●				●		●		●	●	●	
Hydropsychidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●		●	●	●	●	●	●
Philopotamidae	●	●	●	●	●	●	●	●	●		●	●		●				●	●		●		●	●	●	●
Polycentropodidae	●	●		●					●	●	●	●							●						●	
Psychomyiidae	●		●	●			●			●									●				●		●	
Barbarochthonidae	●	●	●	●	●			●	●			●						●	●		●		●	●	●	
Calamoceratidae																										
Glossosomatidae	●	●	●	●			●	●	●			●						●	●				●	●	●	
Hydroptilidae	●	●	●	●	●	●	●	●	●		●	●	●	●	●			●	●		●		●	●	●	●
Hydropsalpingidae				●			●												●						●	
Lepidostomatidae	●	●						●			●	●							●				●	●		●
Leptoceridae	●	●	●	●	●	●	●	●	●		●	●	●	●	●			●	●		●	●	●	●	●	●
Petrothrincidae			●	●								●							●				●	●	●	
Pisuliidae	●	●	●	●					●			●						●	●						●	
Sericostomatidae	●	●	●	●					●			●							●				●	●	●	
Dytiscidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●
Elmidae	●	●	●	●	●	●	●	●	●	●		●	●	●		●		●	●	●	●	●	●	●	●	
Gyrinidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Haliplidae	●	●	●				●					●											●	●	●	
Helodidae	●	●	●	●	●	●		●	●		●	●	●					●	●		●		●	●	●	●
Hydraenidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●		●	●	●	●
Hydrophilidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●
Limnichidae		●	●	●		●			●			●							●		●		●	●	●	●
Psephenidae	●	●	●						●			●							●				●			
Athericidae	●	●	●	●	●	●		●	●			●	●	●				●	●		●		●	●	●	●
Blepharoceridae			●	●									●								●		●	●	●	
Ceratopogonidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Chironomidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Culicidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Dixidae	●	●	●	●	●			●	●	●		●		●	●		●		●	●	●		●	●	●	●
Empididae		●	●	●		●													●				●	●	●	
Ephydridae	●		●	●		●	●			●	●	●					●		●					●	●	
Muscidae	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●		●	●		●		●	●	●	●
Psychodidae		●	●	●	●		●			●	●	●							●	●				●	●	
Simuliidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●		●	●	●	●	●	●
Syrphidae				●																				●	●	●
Tabanidae	●	●	●	●		●	●	●	●	●	●	●	●		●	●	●		●		●		●	●	●	●
Tipulidae	●	●	●	●	●	●		●	●		●	●	●	●			●		●	●	●		●	●	●	
Ancylidae	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●	●	●	●	●	●	●	●
Bulilinae	●	●									●		●						●							
Hydrobiidae			●									●														
Lymnaeidae	●	●	●	●		●	●	●	●		●	●	●	●					●		●		●	●	●	●
Physidae	●	●	●	●	●	●	●	●		●	●	●	●	●				●	●	●	●	●	●	●	●	●
Planorbinae	●	●	●	●	●			●	●		●	●							●		●	●		●	●	
Thiaridae	●		●				●				●									●	●			●	●	
Viviparidae											●															
Corbiculidae		●			●						●								●				●			
Sphaeridae		●				●					●								●				●			●
Unionidae	●										●						●									●

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Table A.2 (Continued): The occurrence of Freshwater Macroinvertebrate taxa per level II Ecoregion.

Taxon	24.01	24.02	24.03	24.04	24.05	24.06	25.01	25.02	25.03	26.01	**26.02**	**26.03**	**26.04**	**26.05**	**27.01**	**28.01**	**29.01**	**29.02**	**30.01**	**31.01**	**31.02**
Porifera										■				■		■		■			
Coelenterata																					
Turbellaria	■	■	■	■	■	■		■		■	■	■		■			■	■		■	■
Oligochaeta	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Hirudinea		■	■	■	■	■		■		■	■	■		■		■	■	■		■	■
Amphipoda	■		■	■	■	■		■													
Potamonautidae		■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Atyidae									■	■	■	■		■		■	■	■		■	■
Paleomonidae																				■	
Hydracarina	■	■	■	■	■	■	■	■			■	■					■	■	■	■	■
Notonemouridae		■		■	■	■														■	
Perlidae												■		■		■	■	■		■	■
Baetidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Caenidae		■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Ephemeridae																				■	■
Heptageniidae				■	■	■				■	■	■		■		■		■	■	■	■
Leptophlebiidae		■		■	■	■		■		■	■	■		■		■		■	■	■	■
Oligoneuridae																■		■			
Polymitarcyidae																				■	
Prosopistomatidae										■				■				■		■	
Telagonodidae					■	■															
Trichorythidae				■	■	■		■		■		■		■		■		■	■	■	■
Calopterygidae																				■	
Chlorocyphidae			■	■						■				■		■		■	■	■	■
Chlorolestidae			■		■	■		■		■						■		■		■	
Coenagrionidae	■	■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Lestidae			■							■								■			■
Platycnemidae			■	■		■												■			■
Protoneuridae				■		■								■				■			
Aeshnidae	■	■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Corduliidae		■	■	■	■	■		■		■	■	■		■		■		■	■	■	
Gomphidae	■	■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Libellulidae	■	■	■	■	■	■		■		■	■	■		■		■		■	■	■	■
Pyralidae		■	■	■		■														■	
Belostomatidae	■		■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Corixidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Gerridae		■	■	■	■	■	■	■		■				■		■		■	■	■	■
Hydrometridae			■	■	■	■		■				■				■		■		■	■
Naucoridae			■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Nepidae			■	■	■	■		■			■			■		■		■		■	■
Notonectidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Pleidae	■	■	■	■	■	■		■		■	■	■				■	■	■	■	■	■
Veliidae	■	■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Corydalidae					■	■															
Sialidae			■			■															
Dipseudopsidae				■	■																■
Ecnomidae			■	■	■	■		■						■		■		■			
Hydropsychidae		■	■	■	■	■		■		■	■	■		■		■	■	■	■	■	■
Philopotamidae					■	■		■											■	■	■
Polycentropodidae														■				■		■	
Psychomyiidae						■												■			
Barbarochthonidae		■	■	■	■	■															
Calamoceratidae																					
Glossosomatidae				■	■	■															
Hydroptilidae		■	■	■	■	■		■			■			■		■		■	■	■	■
Hydropsalpingidae																					
Lepidostomatidae								■						■							
Leptoceridae		■	■	■	■	■	■	■		■				■		■		■		■	■
Petrothrincidae						■															
Pisuliidae						■												■			
Sericostomatidae					■	■		■												■	
Dytiscidae	■	■	■	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■
Elmidae	■	■	■	■	■	■	■	■		■	■	■		■		■		■	■	■	■
Gyrinidae		■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Haliplidae			■	■	■	■													■		
Helodidae		■	■	■	■	■										■					■
Hydraenidae		■	■	■	■	■	■			■	■	■				■	■	■	■		■
Hydrophilidae	■	■	■	■	■	■	■	■		■	■	■		■			■	■	■	■	■
Limnichidae					■	■															
Psephenidae						■														■	■
Athericidae		■	■	■	■	■		■										■		■	■
Blepharoceridae						■														■	
Ceratopogonidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Chironomidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Culicidae	■	■	■	■	■	■	■	■		■	■	■				■	■	■	■	■	■
Dixidae		■	■	■	■	■	■	■			■	■						■			
Empididae				■	■	■						■						■			
Ephydridae	■	■	■	■	■	■						■						■			■
Muscidae		■	■	■	■	■		■		■		■		■		■	■	■	■	■	■
Psychodidae	■		■	■	■	■								■				■			■
Simuliidae	■	■	■	■	■	■	■	■		■	■	■		■		■	■	■	■	■	■
Syrphidae			■	■	■	■															■
Tabanidae		■	■	■	■	■	■	■				■		■		■	■	■	■	■	■
Tipulidae		■	■	■	■	■		■						■		■	■			■	
Ancylidae		■	■	■	■	■		■		■	■	■		■		■		■	■	■	■
Bulilinae			■								■					■	■	■			
Hydrobiidae			■		■																
Lymnaeidae			■	■	■	■		■		■	■	■		■		■	■	■		■	■
Physidae	■	■	■	■	■	■		■		■	■	■		■		■	■	■		■	■
Planorbinae			■		■	■						■		■		■	■	■	■	■	■
Thiaridae	■		■		■	■		■				■					■	■			■
Viviparidae																					
Corbiculidae					■	■				■				■		■		■		■	■
Sphaeridae					■	■				■	■							■	■	■	■
Unionidae	■			■				■				■		■		■		■			■

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